“VALUE OF ROBINSON’S SCORING SYSTEM AND AgNOR SCORE IN CLASSIFICATION OF PROLIFERATIVE & MALIGNANT EPITHELIAL BREAST DISEASES ON FNAC”

DISSERTATION SUBMITTED FOR

M.D. DEGREE EXAMINATION

BRANCH III PATHOLOGY

OF

THE TAMILNADU DR.M.G.R. MEDICAL UNIVERSITY

CHENNAI

TIRUNELVELI MEDICAL COLLEGE HOSPITAL

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CERTIFICATE

This is to certify that the Dissertation **"VALUE OF ROBINSON'S SCORING SYSTEM AND AgNOR SCORE IN CLASSIFICATION OF PROLIFERATIVE & MALIGNANT EPITHELIAL BREAST DISEASES ON FNAC"** presented herein by **Dr. FRANCIS ASIR JOSEPH . J** is an original work done in the Department of Pathology, Tirunelveli Medical College Hospital, Tirunelveli for the award of Degree of M.D. (Branch III) Pathology under my guidance and supervision during the academic period of 2010 - 2013.

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CERTIFICATE

I hereby certify that this work embodied in the dissertation **"VALUE OF ROBINSON'S SCORING SYSTEM AND AgNOR SCORE IN CLASSIFICATION OF PROLIFERATIVE & MALIGNANT EPITHELIAL BREAST DISEASES ON FNAC"** is a record of work done by **Dr. FRANCIS ASIR JOSEPH . J** in the Department of Pathology, Tirunelveli Medical College, Tirunelveli, during his postgraduate degree course in the academic period 2010-2013. This work has not formed the basis for any previous award of any degree.

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This is to certify that the Institutional Ethical Committee of this College unanimously approves the Thesis /Dissertation/ Research Proposal submitted before this committee by Dr. **J.FRANCIS ASIR JOSEPH,** *a* **MD POSTGRADUATE IN PATHOLOGY** *in the Department of* **PATHOLOGY,** *of Tirunelveli Medical College /Hospital, Tirunelveli titled* **"VALUE OF ROBINSONS SCORING SYSTEM AND AGNOR SCORE IN CLASSIFICATION OF PROLIFERATIVE EPITHELIAL BREAST DISEASES ON FNAC."** *registered by the* IEC *as* **065/PAT**/IEC/2010 *dated.* **25.02.2011.** *The Investigator is hereby advised to adhere to all the stipulated norms and conditions of this ethical committee.*

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DECLARATION

I solemnly declare that the dissertation titled **"VALUE OF ROBINSON'S SCORING SYSTEM AND AgNOR SCORE IN CLASSIFICATION OF PROLIFERATIVE & MALIGNANT EPITHELIAL BREAST DISEASES ON FNAC"** is done by me at Tirunelveli Medical College Hospital, Tirunelveli.

The dissertation is submitted to The Tamilnadu Dr. M.G.R. Medical University towards the partial fulfilment of requirements for the award of M.D. Degree (Branch III) in Pathology.

Place: Tirunelveli

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ABBREVIATIONS

AgNOR	: Argyrophilic Nucleolar Organizer Region
DNA	: Deoxy ribonucleic acid
DPX	: Di-N-Butyle Phthalate in Xylene
FNAC	: Fine Needle Aspiration Cytology
H&E	: Hematoxylin and Eosin
mAgNOR	: Mean Argyrophilic Nucleolar Organizer Region
NCI	: National Cancer Institute
NOR	: Nucleolar Organizer Region
NORAP	: Nucleolar Organizer Region Associated Protein
NOS	: Not Otherwise Specified
NST	: No Special Type
pAgNOR	: Proliferative Argyrophilic Nucleolar Organizer Region index
PCNA	: Proliferating Cell Nuclear Antigen
RBC	: Red Blood Corpuscle

rDNA	: Ribosomal Deoxyribonucleic acid
RNA	: Ribonucleic acid
rRNA	: Ribosomal Ribonucleic acid
SAPA	: Subjective Argyrophilic Nucleolar Organizer Region Pattern Assessment
SBR	: Scarff-Bloom-Richardson
S.D.	: Standard Deviation
sig	: Significant
SnRNP	: Small Nuclear ribonucleoprotein
SPF	: S Phase Fraction
TDLU	: Terminal Duct Lobular Unit

CONTENTS

INTRODUCTION

Breast carcinoma is one of the most common non-skin malignancies and is the leading cause of death in women, comprising about 22.9% of all cancers in woman worldwide[1]. However, non-cancerous conditions of the breast are far more common than carcinomas. The breasts are composed of specialized epithelium and stroma that gives rise to both benign and malignant neoplasm. The incidence of female breast cancer is rising rapidly between the age group of 35 to 50 years worldwide. Invasive ductal carcinoma comprises the largest group of malignancy constituting about 65% - 80% of all breast carcinomas[2].

The advent of the medical science has conquered many of the infectious diseases that formerly destroyed large populations of the mankind, but cancer still remains as an unconquerable disease of the non-epidemic kind. It’s ironic and tragic that lesions arising from the breast, readily accessible to self-examination and clinical diagnosis, continue to take a heavy toll on the female gender. Clinical examination, mammographic imaging studies and fine needle aspiration cytology have traditionally been used as a triple assessment tool for evaluation and diagnosis of the breast lesions[2].

Two cytological methods that have been used to obtain material from breast lesions are aspiration of secretions from the nipple and Fine

Needle Aspiration Cytology of breast lesions. Aspiration cytology of nipple secretions is only of a limited use, whereas the application of fine needle aspiration undoubtedly has a major influence on the evaluation and diagnosis of the breast lesions[2].

Fine needle aspiration offers a rapid and safe diagnostic approach which is usually being employed as an outpatient procedure without any need for anaesthesia. In the experienced hands this technique is highly reliable and promptness of diagnosis alleviates patient anxiety and allows time to plan for definitive management. The average sensitivity of this procedure is about 87%, the specificity and positive predictive values are nearly close to 100%, while the negative predictive value still hovers around 60%[2].

Even then the lesions of the breast pose various diagnostic difficulties in distinguishing benign from malignant lesions. Since most of the malignant neoplasm show increased proliferative activity, research had been directed towards finding a reliable proliferative marker. Mitotic index forms one of the oldest and reliable proliferation markers since the advent of light microscopy, but its reproducibility has been greatly debated[3]. Since then various such markers has been identified. Nucleolar Organizer Regions (NOR's) forms one of the earliest proliferation marker

discovered and utilised by Ploton et al. in 1986 to distinguish benign from malignant neoplasm, the latter showing more abundant NORs[4].

The AgNOR staining is quiet simple and easily affordable compared to other expensive and complex procedures like enumeration of S phase fraction (SPF), Proliferating Cell Nuclear Antigen (PCNA), Ki - 67 index and MIB – 1 index. “Earlier the diagnosis of the lesion better is the prognosis”. Having this concept in our mind, our present study has been conducted to assess the accuracy of AgNOR as a proliferation marker in differentiating benign and malignant epithelial breast lesions on Fine Needle Aspiration Cytology.

AIMS AND OBJECTIVES

1) To assess the value of AgNORs in differentiating benign from malignant neoplasm of breast.
2) To evaluate the ability of AgNOR score in differentiating benign proliferative lesions from non-proliferative lesions of breast.
3) To correlate the value of AgNOR score with Robinson's cytological scoring system with regard to malignant lesions of the breast.
4) To evaluate the usefulness of AgNORs as a proliferation marker and a prognostic indicator of neoplastic cells.

REVIEW OF LITERATURE

The human female breasts are modified sweat glands forming a conical projection between the subcutaneous tissues and the pectoral muscles. The upper outer quadrant contains about 40 to 50% of mammary tissue[5]. The mammary tissue is divided into 10-15 ill-defined lobes, which are pyramidal in shape with its apex at the areola. The glandular tissue of each lobes drain into the single collecting duct forming subareolar dilatation known as the lactiferous sinuses before emerging at the nipple. Along the length of these ducts arise the lobular units embedded in a loose connective tissue stroma. Both the glandular and the stromal component give rise to a variety of benign and malignant neoplasm of breast (Fig 1). Both the ducts and the lobular units are lined by a single layer of cuboidal to columnar epithelium surrounded by a basket weave array of myoepithelial cells. Lymphatic drainage of the breast tissue lateral to the nipple is towards the axillary group of nodes while those breast tissues medial to that of the nipple is towards the internal mammary group of nodes[5].

There is no discernible variation between the male and female breast tissues from the time of conception until puberty. While at puberty females exhibit branching and further lengthening of their ducts accompanied by lobular development and proliferation of fibrous stroma

and fat tissues reaching their maximum breast development by the age of 20 years[6,7]. The menstrual cycle is accompanied by minor variations of the breast tissue, but major physiological changes of the breast tissue are seen during pregnancy and lactation. There is major regression of the breast tissue during menopause which merges with aging associated atrophy of the breast.

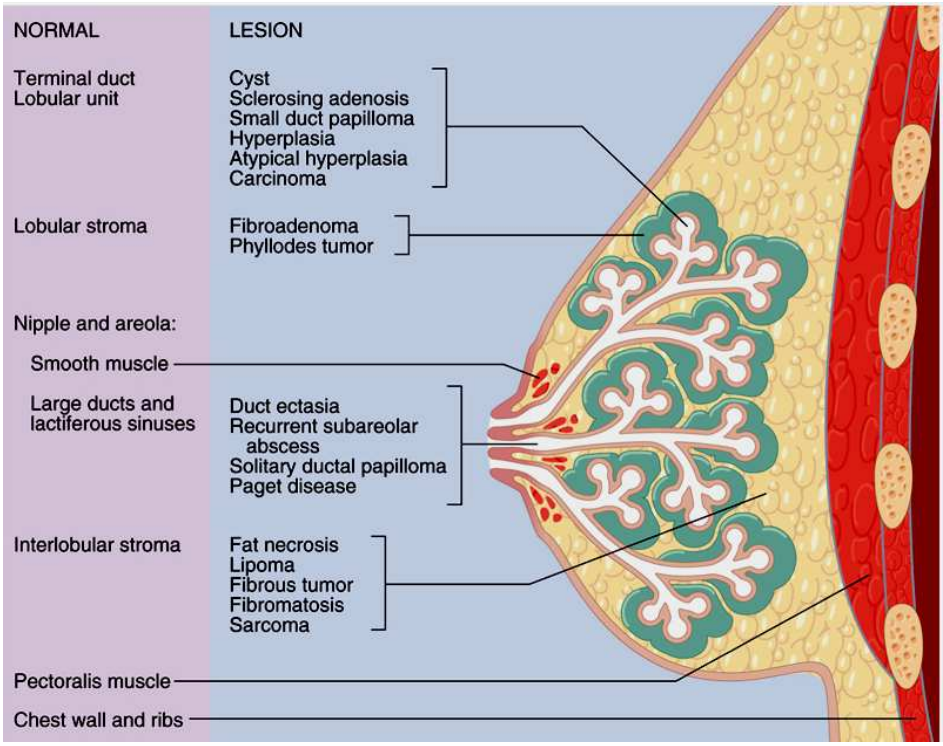


FIGURE 1 Anatomical origin of common breast lesions[8].

These physiological changes at the various age groups give radically different histological appearances. These changes should be differentiated and compared with the pathological processes.

BENIGN EPITHELIAL LESIONS

A wide variety of benign lesions arising from both the ducts and lobules have been observed in the breast. These include non-proliferative changes, proliferative breast lesions and proliferative breast diseases with atypia.

NON-PROLIFERATIVE CHANGES

This group includes wide variety of lesions including duct ectasia, simple cysts of breast, apocrine metaplasia, fibrosis and adenosis of the breast. All these are grouped under the term fibrocystic changes of breast. These lesions generally show fragments of usual duct epithelial cells with a background composed of cyst fluid and cyst macrophages along with scattered bare bipolar nuclei.

CYSTS OF THE BREAST

Single or multiple cysts of various sizes are the most common cause of palpable breast swelling. Cysts are the manifestation of fibrocystic disease and are lined by a single layer of cuboidal or flattened epithelium, occasionally forming papillary projections. The lumens of the cysts are filled with fluid, usually containing desquamated cells and large cells with vacuolated cytoplasm, known as foam cells (cyst macrophages)[9].

The fluid from breast cysts are fairly rich in vacuolated mononuclear or multinuclear “foam cells” of various sizes, and contains various numbers of benign duct epithelial cells that are often poorly preserved. In cysts with papillary proliferation, the epithelial cells are usually more abundant and larger. Approximately one-third of breast cysts are lined by large cells, referred to as apocrine metaplasia cells which contain numerous coarse granules which impart an eosinophilic appearance to the cytoplasm.

FIBROCYSTIC DISEASE

Fibrocystic disease is the commonest disorder of the female breast. It occurs in the mature woman, particularly in the pre-menopausal years. It simultaneously involves the ductal, lobular and stromal elements of the breast. The disorder is also variously referred to as cystic mammary dysplasia, benign mammary dysplasia, fibroadenosis, and benign cystic mastopathy. The affected ducts show areas of dilatation (duct ectasia), which may appear as cyst containing fluid (cyst formation). The lobular ductules undergo hyperplastic proliferation (adenosis) and are surrounded by proliferating stroma (fibrosis). In one variant of fibroadenosis, the hyperplastic ductules are separated and compressed into tubular shapes by bands of dense fibrous tissue (sclerosing adenosis)[10]. Hypertrophy and multiplication of the lining epithelium may also occur (epitheliosis). The

key features to its diagnosis include usually scanty cohesive sheets of benign duct epithelial cells forming a honeycomb pattern with variable number of benign apocrine cells and foam cells in the background of scattered naked bipolar nuclei[11]. Necrotic material may be present in cases with marked dilatation of ducts and the foamy macrophages show inspissated secretions with dark-staining nuclei and a “dirty” appearance of the cytoplasm[12].

The cellular presentation of fibrocystic disease is variable. Dense fibrous tissue is resistant to aspiration and when fibrosis is the predominant component, the sample may be virtually acellular. In cases of marked adenosis, the smear may show only epithelial cells. The cytological diagnosis is therefore necessarily incomplete and as such fibrocystic change is not a specific cytological diagnosis. Wellings and Alpers[13] stated that patients in the age group of 13 to 19 years showed no apocrine metaplasia whereas this change was identified in over half of those above 30 years.

PROLIFERATIVE BREAST LESIONS

These are again subdivided into two groups proliferative breast diseases without / with atypia. Under the category of proliferative disease without atypia comes the moderate to florid epithelial hyperplasia (epitheliosis), complex sclerosing lesion (radial scar), papilloma,

sclerosing adenosis. While under the category of proliferative disease with atypia comes the atypical ductal / lobular hyperplasia.

EPITHELIAL HYPERPLASIA

Normally the ducts and lobules are lined by double layer of epithelial and myoepithelial cells. The epithelial hyperplasia is defined as the presence of more than two layers of cells which can be from either luminal or myoepithelial cell type and these hyperplastic cells tend to distend and occlude the lobules and ducts and even cause distortion of their shape resulting in formation of irregular lumens.

Cytology wise, they are cellular smears with large slightly disorganised sheets of cohesive duct epithelial cells with a tendency of these cells to be arranged in a ‘streaming’ pattern with focal crowding and overlapping of the nuclei, with mild or absent nuclear atypia in a background of few scattered bare bipolar nuclei and a few foamy cells[14].

ATYPICAL DUCTAL HYPERPLASIA:

Atypical ductal hyperplasia has a high cellular proliferation resembling that of low-grade ductal carcinoma in situ and there is a considerable overlap between these two entities and there is no single feature that can be confidently used to differentiate between them[15].

Cytology wise, they are highly cellular smears with large sheets of cohesive mildly atypical epithelial cells with many holes indicating a

cribriform pattern with occasional naked bipolar and myoepithelial nuclei in a background showing necrotic debris and calcium granules.

DUCT PAPILLOMA

These are a group of breast neoplasm that may cause significant diagnostic problems. The main secretory ducts are the most common sites of these neoplasm and they usually present with bloody nipple discharge. The epithelium lining a cystically dilated duct hypertrophies and forms papillary ingrowths into the cyst cavity which are seen as complex branching epithelial sheets and finger like projections lined by epithelial cells showing mild nuclear atypia with a dense fibrovascular stromal core and are seen along with macrophages and variable amount of cystic fluid in a background showing sparse naked bipolar nuclei[16]. Benign duct papillomas may be single or even multiple in number[17].

INVASIVE DUCTAL CARCINOMA

Galen in 2nd century A.D described breast cancer as "The breast carcinoma exactly resembles the animal crab. In this disease, the veins extending out from the unnatural growth take the shape of a crab's legs[18]" Rosen[19] (1979) stated that invasive ductal carcinoma accounted for 75% of breast cancer deaths. Current opinion is that the Terminal Duct Lobular Unit (TDLU) is the site of origin of both invasive ductal as well as lobular carcinoma.

Azzopardi[20] stressed the work of Wellings and colleagues on the point of view that most ductal carcinoma arises in the TDLU. Most malignant tumours of the breast arising from ductal or lobular epithelium are adenocarcinomas. The greater majority by far are invasive ductal carcinomas (80%). Invasive ductal carcinoma of No Special Type (NST) constitutes the majority of about 75%[21].

Cytology wise they are highly cellular with neoplastic cells being arranged in irregular dyscohesive aggregates or sheets with large pleomorphic cells with malignant nuclear features in a background of nuclear debris and granular calcium[22].

The other special categories of primary breast carcinoma include lobular carcinoma, mucinous carcinoma, tubular carcinoma, medullary carcinoma, papillary carcinoma, clear cell carcinoma, secretory carcinoma, adenoid cystic carcinoma and metaplastic carcinoma.

FINE NEEDLE ASPIRATION CYTOLOGY

Era of modern diagnostic Fine Needle Aspiration Cytology (FNAC), which has now become an integral part of the pre-operative diagnosis of any accessible lesions, began with the works and publications of G. Papanicolaou and H. Traut in the early 1940's on cytopathological diagnosis of uterine carcinomas[23]. Diagnostic cytology had resurgence in Europe and particularly in Scandinavian countries

during the 1950s and 1960s, where it thrived before spreading all over the world[24]. However, histopathological basis for interpretation of cytology samples were established only around 1960 by Koss[25]. In a study conducted by Russ about one in eight breast carcinomas diagnosed with FNAC were initially considered to be benign lesions on clinical examination[26].

In 1986 Grant[27] studied from the summary of 18 reported cases the following statistics regarding FNA biopsy

Specificity	- 99.8%
Sensitivity	- 92.5%
Accuracy	- 96.5%
Positive predictive value	- 99.7%
Negative predictive value	- 94.2%

In a study done by Kaninsky[28] (1984) demonstrated that FNAC was an excellent, cost effective diagnostic modality and it may reduce the cost of diagnosis by as much as 90% compared with excisional biopsy and its requirement for hospitalization.

FNAC was initially conceived as a means to confirm the clinically suspicious diagnosis without subjecting the patient to unnecessary surgical interventions[29]. As a preoperative diagnosis it has several advantages by relieving patient anxiety through its rapid diagnosis,

offering time for planning in advance for definitive treatment and also in preoperative staging of the cancers.

NATIONAL CANCER INSTITUTE (NCI) – GUIDELINES[30]

The NCI has given guidelines for a uniform approach to breast FNA biopsy. It recommends the use of 22-25G needle with the needle being aimed at the centre of the lesion and moved in different directions within the lesion. For necrotic, partly cystic and fibrotic lesions the needle should be aimed at the rim of the lesion and sampling should be done just within the rim tangentially. Small lesions are better stabilized by bringing it to an immobile position under the skin before sampling. An average number of 2-4 needle passes is recommended for adequate sampling. However more passes are required when encountered with following conditions like when the tumour is difficult to stabilize or penetrate, a dry tap, larger tumour of more than 4cms.

Adequacy: Adequacy is assessed by two judgments: First is the opinion of the aspirator that the report of the cytological findings were consistent with the clinical findings, second is the pathologist opinion that the cytology smears do not have any significant artefacts or distortion.

There are no specific requirements for minimal number of duct epithelial cells to be present for adequate sampling. The NCI guideline

also recommends the usage of Robinson's grading system for grading of the breast carcinomas.

CYTOLOGICAL GRADING - ROBINSON'S GRADING SYSTEM:

Robinson et al[31] developed a protocol in 1991 for the cytological grading of invasive ductal carcinoma - Not Otherwise Specified (NOS). This method has three cytological grades which correlated well with the histological grading (Bloom and Richardson histological grading system). This method is simple, quick and easily reproducible method. In the Robinson's system of grading for carcinomas of breast, six different cytological parameters were applied, namely cell dissociation, uniformity of the cell, cell size, nucleolus, nuclear margin and nuclear chromatin. A score of 1-3 is given to each one of these parameters and the tumour is graded by adding up all the individual scores[32].

Robinson et al. established three cytological grades namely grade I, grade II and grade III as depicted in table 1.

TABLE 1 ROBINSON'S GRADING SYSTEM[31,32]

Criteria	Score		
	1	**2**	**3**
Cell dissociation	Mostly in clusters	Mixture of single cells and cells in clusters	Mostly single cell
Cell uniformity	Monomorphic	Mildly pleomorphic	Pleomorphic
Cell size	1-2 times RBC size	3-4 times RBC size	>5 times RBC size
Nuclear margin	Smooth	Folds	Buds and clefts
Nucleoli	Indistinct	Noticeable	Abnormal
Chromatin	Vesicular	Granular	Clumped and cleared

Final score ranges from 6 – 18 and these were graded below as

Grade I : Score 06 – 11

Grade II : Score 12 - 14

Grade III : Score 15 - 18

The accuracy of Robinson's grading method was 83%, true positivity was 77.33% and false negativity was 11.33%[33].

Mouriquand J et al.[34] applied a cytological grading method for FNAC smears of breast. Both Topographic and Nuclear Criteria were

given more importance in this method. Three grades of classification of tumours were followed. The salient features of grading system were

TABLE 2 GRADING SYSTEM OF MOURIQUAND et al

Criteria	**Grade 1**	**Grade 2**	**Grade 3a**	**Grade 3b**
Cells	In clusters	Both clusters and isolated cells	Predominantly isolated cells	
Shape & size	Uniform	Larger	Anisonucleosis	
Chromatin	Regularly distributed	Irregularly distributed	Highly dispersed	
Cytoplasm	-	-	Absent	Well preserved
Nucleoli	-	Enlarged blue	Bright red	Bright red with a surrounding clear halo
Mitosis	-	-	-	Numerous

This Mouriquand's grading method has an accuracy of 77%, true positivity of 69.33% and false negativity of 15.33%[33]. Mouriquand's grading method[34] also corresponded well with that of the Bloom and Richardson grading system and had a good positive prognostic correlation. However, this method had not gained wide acceptance. In a study comparing the two methods with that of histological grading[35], the diagnostic accuracy and sensitivity of both Robinson's and Mouriquand's

methods were similar. However, Mouriquand's method had a low specificity.

The Robinson's method of cytological grading was far more specific when Bloom and Richardson method of histological grading was considered as gold standard. The criteria for grading[32] tumour by the Robinson's grading system were simpler and easier to reproduce as compared to the Mouriquand's grading system. The other grading systems of importance are Hunt's et al grading system which has an accuracy of 70.66%, true positivity of 70.66% and false negativity of 29.33% and the Howell (SBR) Grading System which has an accuracy of 53.89%, true positivity of 40% and false negativity of 31.25%.

Compared with other cytological grading systems Robinson's grading system had a correlation of about 83% with Bloom and Richardson histological grading method and the sensitivity and specificity were highest by Robinson's cytological grading system. The Robinson's method of cytological grading was also far more specific when Bloom and Richardson histological grading method was considered as gold standard[32].

Dutta et al[36] (2001) studied fine needle aspiration cytology of 51 cases of breast masses out of which 28 turned out to be malignant and

the remaining were benign lesions composed of fibrocystic disease, mastitis and fibroadenoma. Diagnostic accuracy of FNAC was 90.2%.

Chaiwum et al[37] (2002) studied Fine Needle Aspiration Cytology of 2375 cases from 1994 to 1999, benign lesions accounted for 48%, 5% were suspicious for malignancy, malignant neoplasm accounted for 15%, 32% were unsatisfactory smears. FNAC showed a specificity of 99.5% and sensitivity of 84.4%.

Young et al[38] (2002) reported that aspiration cytology of carcinomas of the breast were rightly identified as malignant and the values of each of the subtypes were - ductal carcinoma - 65%, for lobular carcinoma it was 20%, for mucinous variety it was 27% and for medullary carcinoma it was 12%. This study showed that Fine Needle Aspiration Cytology is the most reliable method for breast carcinoma diagnosis.

HISTOLOGICAL GRADE

Bloom and Richardson histological grading method is the most widely used method for grading of the breast carcinoma and this method is easily reproducible, quite simpler and gives good positive prognostic implications. This system grades breast carcinoma into three histological types based on measurement of tubular differentiation, nuclear pleomorphism and proliferative activity (mitotic figures)[39].

TABLE 3 BLOOM AND RICHARDSON HISTOLOGICAL GRADING SYSTEM[39]

Parameter	Criteria	Score
Tubule Formation	> 75%	1
	10-75%	2
	< 10%	3
Nuclear Pleomorphism	Small and uniform	1
	Moderate variation	2
	Marked variation	3
Mitotic count / 10hpf	0 - 5	1
	6 - 10	2
	>11	3

The breast carcinomas were graded into 3 grades after summing up all the score of the three features and values range from 3 – 9**.**

TABLE 4 BLOOM AND RICHARDSON HISTOLOGICAL GRADING SYSTEM[39]

Grades	Score
Grade 1 (well differentiated)	3 – 5
Grade 2 (moderately differentiated)	6 – 7
Grade 3 (poorly differentiated)	8 – 9

AgNORs: ARGYROPHILIC NUCLEOLAR ORGANIZER REGIONS

The Nucleolar Organizer Regions (NORs) are loops of DNA projecting into the nucleoli of interphase nuclei[40]. These are specific portions of DNA (deoxyribonucleic acid) called rDNA (ribosomal DNA) that, by using the enzyme RNA (ribonucleic acid) polymerase-1, codes for the transcription of ribosomal RNA (rRNA) which in turn results in the synthesis of proteins by the cell. Since NORs encode for ribosomal RNA a necessary step in protein synthesis (Fig 2), it has been suggested that they correlate with cellular proliferative activity[41]**.** Organization of a typical Nucleolar Organizer Region (NOR) is shown in figure 2.

Nucleolar Organizer Region (NOR) are DNA loops that are located at the ends of each of the acrocentric chromosomes 13, 14, 15, 21 and 22[40]. NORs occur in pairs on the five acrocentric chromosomes (one on each of the daughter chromatid), and at the metaphase potentially 20 NORs could be seen. However, in diploid cells visualising ten NORs can be regarded as a full complement, as the metaphase is a very transient phase.

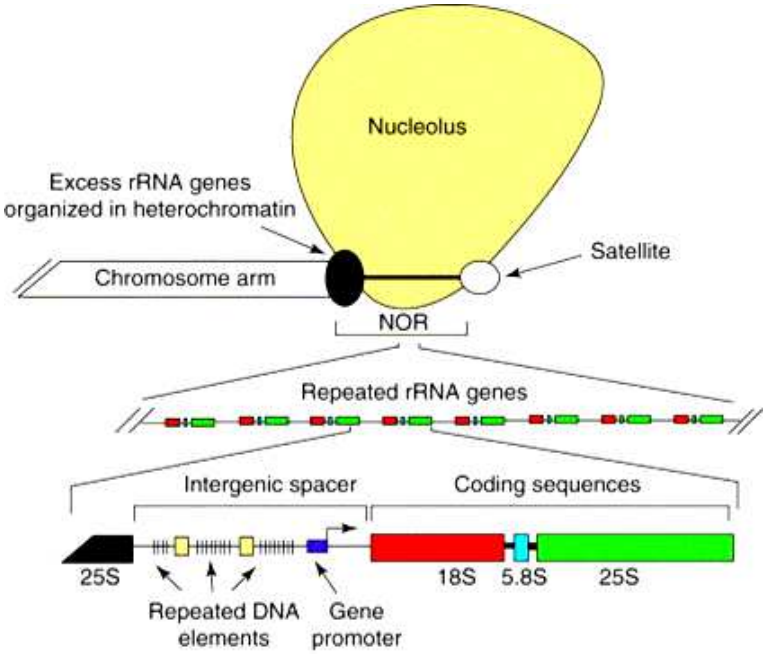


FIGURE 2 Organization of a typical Nucleolar Organizer Region[41]

PHYSIOLOGY OF AgNOR'S AND CELLULAR KINETICS:

Ultra structure of the human nucleoli shows three substructures[42]**.** These are composed of

1. The dense fibrillar component – packed electron dense fibrils
2. The fibrillar center – composed of loose network of fibrils.
3. The granular component.

The dense fibrillar component consists of lightly packed electron dense 3-5nm thick fibrils and is the site for processing of ribosomal RNA precursors and stains with antibodies targeted to 'fibrillarin', which is a protein associated with U3 small nuclear ribonucleoprotein (SnRNP)[41].

The fibrillar center is the site for the production of ribosomal RNA and it contains topoisomerase I, RNA polymerase I and ribosomal DNA.

It is equivalent to that of interphase NOR visualised by the light microscopy.

The granular component is composed of particles of ribosome precursor.

Tumour behaviour mainly depends upon the Cellular kinetics. The behaviour of the tumour can be assessed by determining the proliferation rate. The cell cycle can be divided into four phases based on the nuclear chromatin activity. They are S, G1, G2 and G0 phases. There is a short resting phase of the cell undergoing replication at the ‘S’ phase. Thus, the DNA content at the end of ‘S’ phase is an indicator of proliferative activity and AgNOR detects the DNA content at this stage[43].

The size, shape and number of the NORs vary according to nucleolar transcription and are related intimately to the cell cycle. Since nucleolar transcription rate and cell turnover is comparatively high in proliferating cells, assessment of the morphology and quantity of NORs helps in assessing cell proliferation.

During the prophase there is dispersion of the components of the fibrillar centre and these structures exist in a constant position at metaphase which are seen on the short arms of five acrocentric chromosomes - 13, 14, 15, 21 and 22[40].

However, the five acrocentric chromosomes have a tendency to associate through the satellite regions of these chromosomes and thus have a greater impact on the number of NORs observed and counted.

DEMONSTRATION OF AgNORs:

The NORs can be visualised by various techniques which either demonstrate NOR associated proteins or the ribosomal DNA itself

TABLE 5 DEMONSTRATION OF NORs[41]

Reagent	Target
Silver colloid (AgNOR)	NORAPs
Bismuth ions	100K NORAP
Radiolabelled rRNA	rDNA
Antibodies	NORAP epitopes

The most popular and the simpler method among all these in identifying the NORs is the silver staining technique. The structures demonstrated by this method are called AgNORs (Argyrophilic Nucleolar Organizer Regions). This silver staining technique identifies neither the rRNA nor the rDNA, but the acidic NORAPs (Nucleolar Organizer Region Associated Proteins) seen in association with the site of RNA transcription.

AgNORs are usually aggregated tightly within one or two nucleoli in a normal cell, as seen in cytological smears and individual AgNORs

are often not discernible. The number of AgNORs detected depends upon several of these factors[44]. These include-

1. The stage of the cell cycle.

2. The number of NORs bearing acrocentric chromosomes in their karyotype

3. The level of transcriptional activity of the cell.

There is thus a remarkable difference between AgNOR counts in chromosome spreads and those observed in histology sections of the similar cell preparations. Since the AgNORs congregate within a relatively small nucleolus in histological sections, there is a greater difficulty in visualising the individual AgNORs.

In malignant lesions, the AgNORs are dispersed throughout the nucleus to a varying extent, enabling them to be easily visualised by the cytologists. Therefore quantification of the AgNORs in interphase nuclei is probably related more to their dispersion throughout the nucleoplasm than to the actual number present in the nucleus. Therefore 'AgNOR count' in benign and malignant lesions does not denote the absolute number of AgNORs but it is rather a numerical index of dispersion of AgNORs within the nucleus. This connotes that dispersion in itself may reflect the proliferative state of the cell. Hence the number of visible

AgNORs indicates the number of cells in the current phase of transcription[43].

NORs were first visualized in 1975 by means of a simple silver staining technique that recognizes these argyrophilia-associated proteins where they appear as black / brown dots within the nucleus of the cell.

The claims that AgNORs were visualised significantly higher within the nucleus of malignant tumours compared to that of the reactive / benign lesions has attracted a lot of attention for AgNORs being regarded as a cell proliferation marker[43].

An apparent increase in the mean AgNOR count was noticed in the cells under the following conditions:

1. When the cell proliferation was very active, the nucleolar dissociation was present in almost all cells, and that the AgNORs were seen throughout the nucleus.
2. A defect of the nuclear association could result in AgNOR dispersion throughout the nucleus.
3. An increase of the AgNOR bearing chromosomes resulting from increased cellular ploidy.
4. Prominent increase in AgNOR activity is associated with increased transcriptional activity.

In the benign neoplastic cells AgNORs are aggregated within a relatively condensed small nucleus and hence show only 1-2 AgNOR per nucleus, which is been attributed to the difficulty in visualising the individual NORs. While in malignancy, or in conditions of increased cellular proliferation, AgNORs get dispersed throughout the nucleus, enabling the cytologist to enumerate them more easily. Hence, the AgNOR quantification depends mainly on the degree of disaggregation or dispersion of large number of AgNORs within the nucleus of the neoplastic cells.

AgNOR – TECHNICAL ASPECTS

The main advantage of the AgNOR staining technique is that, it is a one-step silver-staining method, which is a relatively simpler staining method, and also has the advantage of the ease of application of this staining method even onto the archival tissues. It can also be used to demonstrate NORs easily on routinely processed cytology smears and histology sections[45]. The principle disadvantage is the time consuming process of counting of the little dots, often associated with inter-observer variations.

In concise form, the one step silver-staining method consist of a mixture of 50% silver nitrate solution and 1% formic acid in 2gms% of gelatin solution which acts as a colloid stabilizer[41]. These solutions have

to be prepared separately and mixed freshly upon use. Cytological smears are incubated in this solution mixture for a period of 45 min to one hour and then washed, dehydrated, cleared and mounted for light microscopy examination. Both the light microscopy and ultra structure studies have implicated that this method is remarkably specific as a means of detecting interphase / metaphase NORs. Staining sequentially with radiolabeled rDNA and rRNA has shown a good correspondence between the binding sites and silver stained NORs in interphase nucleoli and on chromosome spread.

The NORs can be seen as discrete black / brown dots in a pale yellow background at the light microscopy level and can be enumerated using an oil immersion lens. Counts in 50-100 neoplastic cells are usually made and the results are expressed as a mean number of AgNORs visualised per nucleus. Lymphocytes are usually employed as internal controls.

This technique can be used with success after making minor modifications, in both semiautomatic and automatic image analysis hardware[46]. In this technique the total amount of AgNORs per nucleus is measured, rather than the number of sites counted.

The intensity of the AgNOR staining is observed to be dependent on the fixation regimen employed and results vary from one fixative to

the other. Alcohol based fixatives, Carnoy's fixative and 95% ethanol gives optimal results. Mercury and dichromate fixatives are highly detrimental to the AgNOR staining[47].

AgNOR STAINING REACTION & PROBLEMS:

The silver staining technique is based upon the principle that, the silver salts as a result of their high electron charge density and by virtue of their phosphate moieties have a high affinity for the acidic NORAPs.

Generally, the AgNOR silver staining method is been run for about one hour irrespective of whether cytological smears or histological sections were stained, but recently minor alterations have been put forth to reduce the staining time and also to incorporate internal controls to allow counting of subsidiary AgNOR dots.

First, the persistent problem with the usage of any kind of silver staining method is the non-specific silver grain deposits in the background. Usage of clean glassware and very pure deionised water can overcome this background staining. However, recently certain minor modifications in the AgNOR staining method have been put forth that can overcome this problem of background staining. These are:

1. Usage of an inverted incubation technique, where the slides are inverted into the staining solution. This technique helps in maintaining a high degree of contrast between the background and the AgNORs[48].

2. Glycine blocks both free and reversibly bound aldehyde residues left over by formalin fixation, thereby reducing the background silver precipitation. Hence, pre-incubation with glycine prior to AgNOR staining can reduce the background stain.
3. After completion of the staining procedure immersing the slides in a 10% nitric acid solution can minimise the background stain.
4. Replacement of gelatin by polyethylene glycol as a protective colloidal developer medium[49].

Second, the intensity of staining varies considerably even with slight variations of the staining time; which if over-stained obscures the individually clustered AgNORs within nucleoli, or if under-stained renders them too faint to be assessed.

Third, in the histology sections even minor variations in the thickness of the sections affect the apparent number of AgNORs within the nuclei, thus requiring uniformity in section thickness of around 3 - 4 μm. Application of this technique to cytology smears thus eliminates this problem[50]. Thus AgNOR dot count study on cytological smears has shown to be far more superior compared to those on histological sections. Cytological smears also show a better discriminative value of AgNOR dots compared to those on histological sections[51].

One great advantage of this technique is that, previously stained cytology slides can also be reused for silver staining, thus providing an excellent guide to the diagnosis especially in doubtful cases and when extra-unstained slides are not available.

The major disadvantages are:

1. Inter-observer variation is the major cause of inaccuracy and inconsistency.
2. The counting procedures adopted are usually manual and are prone to subjective variations.
3. Misjudged counts may result due to overlapping of the NORs within the nucleus[43].
4. The dots of AgNOR in the interphase nuclei may not always correspond to the number of such types in the karyotype of the nucleus[52].

MODIFICATIONS IN THE AgNOR TECHNIQUE:

After the AgNOR technique was first described by Ploton in 1986 it has undergone several modifications with an aim to improve the overall staining quality. Some of the modifications that are worthwhile to mention include

First, combination of Feulgen reaction with modified AgNOR staining technique, which not only enables the counting of active NORs

but also the evaluation of the amount of DNA within the same cell nucleus by the Feulgen reaction.

Secondly, combination of AgNOR staining technique with cytofluorometric analysis on cell suspensions.

Thirdly, the use of AgNOR technique along with automatic image analysis software makes these technique far less prone to subjective errors than the traditional methods[46].

AgNOR - ENUMERATION [53]

The types of Nucleolar Organizer Regions within the nucleus can be categorized into three groups.

The first one is the 'Aggregated AgNOR' which are seen as rounded, solitary structures and corresponds to the nucleolus of the cell, this type is often seen in resting cells and lymphocytes and the individual NORs cannot be distinguished within the nucleus of these cells.

The second type is the 'Nucleolar pattern' which is seen in the nucleus of the proliferating cells and the NORs are seen to be dispersed only within the nucleolus of the cell.

Finally, the third type is the 'True AgNORs' that are seen to be dispersed throughout the nucleoplasm and are often seen in highly malignant neoplastic cells. These features can be demonstrated well in the cytological smears[53].

METHODOLOGY:

There are five methods for enumeration of AgNORs based on their count, morphology and distribution. They are

1. Mean AgNOR count
2. AgNOR proliferative index
3. AgNOR size variation grading
4. AgNOR distribution in the nuclei
5. Subjective AgNOR Pattern Assessment (SAPA)

Mean AgNOR count (mAgNOR):

Mean count of the number of NORs present in the nucleus of the 100 neoplastic cells. mAgNOR value correlates with the mean DNA content of the cells indicating the cell ploidy.

AgNOR proliferative index (pAgNOR):

It is the percentage of neoplastic cells exhibiting more than five NORs within the nucleus of the 100 counted cells. pAgNOR value represents the number of cells in the S-phase fraction.

AgNOR size variation and distribution grading:

In 1991 – 1992 Ahsan et al utilised the criteria of size variation and distribution of AgNORs within the nucleus and demonstrated higher variation score of these parameters in malignant neoplasm compared to the benign counterparts.

TABLE 6 AgNORs SIZE VARIATION GRADING

AgNOR Size Variation	Score
More or less uniform	0
Two different sizes	1+
More than two different sizes (but not those of 3+)	2+
All grades and sizes including too minute to be counted	3+

TABLE 7 AgNOR DISTRIBUTION IN THE NUCLEI

AgNOR distribution - nuclei	Score
Limited to nucleoli	0
Occasional dispersion outside nucleoli	1+
Moderate dispersion outside nucleoli	2+
Widely dispersed throughout the nucleus	3+

Subjective AgNOR Pattern Assessment:

Meehan et al proposed a method for scoring of AgNORs called 'Subjective AgNOR Pattern Assessment (SAPA), which was based on morphological patterns, variation in the size and shape of the NORs, and whether they are aggregated or scattered[54].

In a study by Dhakhwa R et al[55] on 110 breast lumps observed the mean AgNOR count in benign breast lesions was 2.63 ± 1.36 while the SAPA score was 6.26 ± 1.19. The SAPA score in malignant breast lesions was10.05 ± 2.22 and the mean AgNOR count was 8.42 ± 2.53

When the cut off score for AgNOR count / nucleus is taken as 6 for malignant neoplasm of breast, then the diagnostic accuracy is 95.5%, specificity is 88.9%, sensitivity is 89.5%; positive predictive value is 82.2% and the negative predictive value is 98.5%.

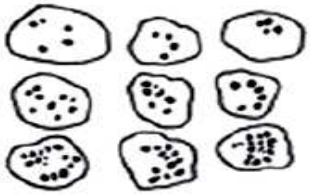
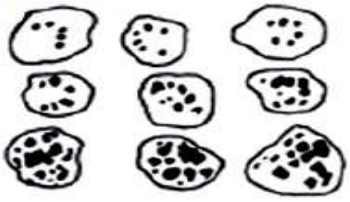
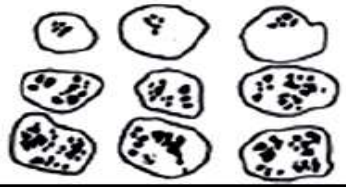
When the cut off value for SAPA score is taken as 8 for malignant neoplasm of breast, then the diagnostic accuracy is 85.5%, specificity is 83.3 %, sensitivity is 89.5%; positive predictive value is 73.9 % and the negative predictive value is 93.8% (Table 7a).

In cases that presented with diagnostic difficulties on FNAC subjective pattern assessment and AgNOR counting showed comparable accuracy in differentiating malignant from benign lesions. In some cases this may give contradictory results and hence more helpful when they are considered together.

TABLE 7a: AgNOR COUNT AND SAPA SCORE IN BREAST LESIONS[55]

Diagnosis	Number of cases	AgNOR Count	SAPA score
Fibrocystic changes	7	2.71+/-1.38	6+/-1.55
Fibroadenoma with fibrocystic changes	7	2.86+/-1.21	5.86+/-3.8
Intraductal papilloma	1	5	7
Infiltrating ductal carcinoma - NOS	32	8.31+/-2.6	9.94+/-2.2

TABLE 8 SUBJECTIVE AgNOR PATTERN ASSESSMENT

Parameter	Score	Illustration
Estimated number per cell		
Few (<5)	1	
Several (5-10)	2	
Many (>10)	3	
Variation satellite size and shape (score each)		
Uniform	1	
Moderate variation	2	
Marked variation	3	
Variation cluster size and shape (score each)		
Uniform	1	
Moderate variation	2	
Marked variation	3	

SAPA score is a more rapid, reproducible and and less time consuming than counting of AgNOR dots[55]. Both SAPA score and AgNOR counts gave similar results on cytology[56].

Khanna AK et al[56] in his study found SAPA score was most useful in differentiating benign neoplasm from malignant neoplasm of breast in both the cytology smears and histology specimens.

AgNOR – ITS APPLICATIONS:

AgNOR as a one step silver colloid staining method was used first in the prostatic specimens. As the years followed AgNOR staining method was performed on a variety of specimens mainly to differentiate malignant from benign lesions. In the malignant neoplasm the increasing AgNOR count correlated well with the tumour aggressiveness.

AgNORS IN BREAST:

Since the time cytogenetic workup studies were performed malignant breast lesions showed unusual and ectopic (Nucleolar Organizer Regions) NOR patterns, this has lead the pathologist to explore the potential of AgNORs in differentiating borderline breast lesions from those of the malignant ones[57]. According to various studies, AgNOR values correlated very well with the prognostic indices like axillary lymph node status, tumor size, Ki-67 index, MIB-1 index and mitotic counts[57].

CYTOLOGY APPLICATIONS:

In the recent past, AgNOR has emerged as a wonderful tool in assessing the proliferative activity of a given lesion in the cytology smears enabling the cytologist to assess the aggressiveness of the neoplasm pre-operatively. Main advantage of AgNORs in cytology smears is that one need not worry about the section thickness as in histology as thicker sections make it difficult to enumerate individual AgNOR dots. While in cytology smears even distribution of cells makes enumeration of AgNOR dots quite simpler[40].

In the study conducted by Roller E et al[58] in 1993 on 56 cases of malignant and 20 cases of benign breast neoplasm, he found a clear difference between benign and malignant neoplasm of breast, the latter showing significantly higher AgNOR counts.

Reddy GS, Sesikeran B, Bhaskaran CS[59] also in the same year conducted a study on ten malignant and benign epithelial neoplasm of breast, and they concluded that quantitative analysis of AgNORs enables one to differentiate benign from malignant lesions.

A prospective study was conducted by Karmakar T, Radhika S, Gupta SK[60] in the year 1995 on the cyotological smears of both benign and malignant breast lesions encompassing proliferative lesions, fibroadenoma, fibrocystic change and ductal carcinoma of breast, found

that the mean AgNOR count of 16.63 in malignant neoplasm was much higher and statistically significant compared to the mean AgNOR count of 6.39 in benign neoplasm. They concluded that a cut-off AgNOR value of 11 can reliably be utilised in differentiating benign from malignant neoplasm. Whereas a study conducted on assessing the number of AgNOR dots in 64 malignant and 31 benign neoplasm of breast on cytological smears by Mehrotra A, Chandra T[61] concluded by putting forth a cut off point of 4 to be reliable indicator to differentiate benign from malignant neoplasm of the breast.

In a study conducted by Simha et al[62] in the year 1996 on the prognostic value of AgNORs in breast neoplasm showed that the AgNOR counts correlated very well with desmoplasia, mitosis and tumour size. Higher NOR counts were seen in ER/PR negative neoplasm.

In a study conducted by Kumar et al[63] in the year 1997 assessed the AgNOR count of breast carcinomas in the cytology smears of 56 cases and found that the AgNOR counts correlated well with stage of the cancer, tumour size, lymph node status and recurrence rate of tumour.

A prospective study was conducted by Hasnan J, Jayaram G[40] on the cytology smears of 31 cases of benign and 25 cases of malignant breast neoplasm with histological correlation in about 26 cases, found that mean AgNOR count ranged from 2.55 to 5.0 in benign neoplasm

and the range in malignant neoplasm was 5.8 to 17.2. The difference in the mean AgNOR count between the benign and malignant lesions was statistically significant. None of the cases showed overlap of mean AgNOR values in the cytological smears.

Khanna AK, Kumar M, Ansari MA, Khanna A[64] studied both cytology and histology of 73 breast lesions which included 27 benign and 46 malignant neoplasm and assessed the correlation between cytology and histology using Subjective AgNOR Pattern Assessment (SAPA) score and mean AgNOR dot counts. They concluded that both SAPA score and mAgNOR counts were useful in differentiating malignant from benign neoplasm in both histology specimens and the cytology smears and both gave similar results. Mean AgNOR count of malignant neoplasm was 6.94 while in benign neoplasm it was 2.75 in Fine Needle Aspiration Cytology. SAPA score of malignant neoplasm was 9.02 and 5.87 in benign neoplasm. They concluded that Subjective AgNOR Pattern Assessment score is more rapid, reproducible and convenient method of AgNOR assessment[64].

Meehan SM, Carney DN, Magee H, Dervan PA[54] evaluated the cytological preparations obtained from surgical specimens for AgNOR count, shape, size and clustering. The malignant lesion had a mean AgNOR count of 9.52 while benign lesion had mean AgNOR count of

4.44, and they concluded that the diagnostic accuracy of combined pattern assessment and counting of NORs was 90% in distinguishing benign and malignant neoplasm. The median score for benign lesions were 7 and for malignant lesions it was 13.

AgNORs being indicators of cellular proliferative activity correlated well with Ki-67 index in a study conducted by Dervan PA, Gilmartin LG, Loftus BM, Carney DN[65] on 70 cases of malignant breast lesions and 27 cases of benign breast lesions. The correlation between Ki-67 scores and AgNOR counts was highly significant. The view of these authors was also shared by Canepa M et al[66] who conducted a study on 53 cases of intra ductal breast carcinoma.

Kesari AL et al[67] evaluated 120 cases of intra ductal breast carcinoma and found a good positive correlation between histological grading, AgNOR score and PCNA expression. Poorly differentiated tumours had a highly elevated AgNOR counts.

Our present study was aimed to find out whether there is any significant difference in the AgNOR values of benign and malignant neoplasm of the breast and also to find out if there is any significant change in the AgNOR values between the non-proliferative and proliferative benign lesions of the breast. The correlation between the Robinson's scoring system and the AgNOR scoring system was also evaluated in malignant neoplasm of the breast.

MATERIALS AND METHODS

This prospective study was conducted in the Cytology Laboratory of the Department of Pathology, Tirunelveli Medical College spanning a period of about 18 months from May 2011 to October 2012. From 423 cases of Fine Needle Aspiration Cytology of breast lesions 100 cases of breast lesions were randomly selected and examined, which included 40 cases of carcinomas and 60 cases of proliferative and non-proliferative breast lesions. This study was conducted after obtaining clearance from the Institutional Ethical Committee.

FINE NEEDLE ASPIRATION CYTOLOGY

FNAC was performed on patients who presented with palpable breast lesions.

INCLUSION CRITERIA:

- All patients presenting with palpable breast lesions.

EXCLUSION CRITERIA:

- Patient who refused FNAC procedure.
- Patients in whom no definable breast mass can be detected
- Cytological smears with air-drying artefact or improper staining

METHOD OF COLLECTION:

After making the patient comfortable the FNAC procedure was explained in detail. The FNA procedure was performed as an outpatient

procedure without any anaesthesia in the cytology laboratory of our department. FNAC was done after disinfecting the skin with alcohol scrub and using a 23 gauge needle with 10ml disposable syringe attached to the syringe holder. The palpable breast lesion was fixed between the thumb and index finger of one hand and with the other hand the needle was inserted to the desired depth within the mass. The material was aspirated under negative pressure with 3 - 4 short passes in different directions. The needle was withdrawn after the negative pressure was released, and the material aspirated was expressed on to glass slides, smeared and fixed immediately in 95% ethanol. The slides were stained subsequently with Hematoxylin and Eosin and AgNOR stain and unstained slides were kept for future examination.

AgNOR staining was performed using a one step silver – colloid technique using a mixture composed of 50 % silver nitrate solution and 2gms% gelatin in 1% Formic acid solution in a ratio of 2:1. These solution mixtures are layered over the slides and are kept in a dark room for a period of 50 – 60 minutes. These slides are then washed, dehydrated, cleared and mounted for examination under the microscope. The detailed AgNOR staining procedure is given in **Appendix – I**. The staining protocol for Hematoxylin and Eosin stain is given in **Appendix – II**.

Cytological grading of breast carcinomas was done according to the Robinson et al. grading system which is a three-tier grading system, dividing carcinomas into grade 1, grade 2 and grade 3. The AgNOR enumeration and analysis of all the smears were done and the observations were noted down by making a master-chart with all the features of the above grading system. Correlations between these grading systems were assessed. For this study, an Olympus microscope with 10X, 40X and 100X magnification objectives and 10X magnification eyepiece was used. The digital images of the selected stained smear preparations were photographed.

ENUMERATING AgNORS:

AgNORs are visualised as blackish or brown dots in a pale yellow background, both in the nucleolus and within the nucleoplasm. The following AgNOR parameters were calculated.

Mean AgNOR count:

The number of AgNORs within the nuclei of 100 neoplastic cells is calculated using a 100X objective. The mean numbers of NORs per nucleus were then calculated and results were expressed as a mean count +/- Standard Deviation.

Proliferative AgNOR index (pAgNOR):

pAgNOR is the percentage of neoplastic cells showing more than five AgNOR dots within the nucleus of the 100 counted neoplastic cells.

Distribution and size variation of AgNORs:

The size variation and the distribution of the AgNORs within the nucleus are evaluated and given a score of 0, 1+, 2+, 3+ according to the criteria given in Table 6, Table 7.

Subjective AgNOR Pattern Assessment (SAPA):

The SAPA scoring of the AgNORs are done based upon the criteria given in Table 8, which takes into consideration the morphological characteristics and whether the NORs are clustered or scattered within the nucleus.

ROBINSON'S GRADING SYSTEM:

Robinsons grading system is used in the grading of malignant neoplasm of breast stained with H & E stain. The criterion for this scoring system is given in Table 1, which includes cellular dissociation, uniformity, size, nuclear margin, nucleoli morphology and chromatin pattern.

Correlation study between the Robinson's score and the AgNOR scoring system for grading malignant neoplasm of breast was conducted. The ability of AgNORs in distinguishing benign (proliferative and non-proliferative) neoplasm from malignant neoplasm of the breast was studied.

OBSERVATION AND RESULTS

This prospective study was conducted in the Cytology Laboratory of the Department of Pathology, Tirunelveli Medical College for a period of 18 months and the following observations were made.

TABLE 9 BREAST FNAC vs OTHER SITES

Duration	Total number of FNAC cases	Number of breast FNAC cases	Percentage
May 2011-December 2011	1622	172	10.6%
January 2012-October 2012	2132	251	11.8%
Total	3754	423	11.3%

In the study period of 18 months duration from May 2011 to October 2012 Fine Needle Aspiration Cytology was performed on a total of 3754 cases. Out of these cases the breast lesions constituted about 423 cases giving a percentage of 11.3%.

172 cases of breast aspiration cytology out of 1622 total aspiration cytology cases were studied during the first 6 months period in 2011 constituting 10.6%. The following 10 month study in 2012 showed 251 breast aspiration cytology cases out of 2132 aspiration cytology cases constituting 11.8%, as shown in Table 9 and Chart 1.

TABLE 10 DISTRIBUTION OF NON-NEOPLASTIC AND NEOPLASTIC LESIONS ON BREAST CYTOLOGY

Breast lesions		**Number of cases**	**Percentage**
Non - neoplastic		32	7.6%
Neoplastic	**Benign**	287	67.9%
	Malignant	104	24.5%
Total		423	100%

Out of the total 423 breast lesions 32 were non-neoplastic comprising 7.6% of the total breast lesions. Out of the remaining 391 neoplastic cases benign lesions were 287 cases constituting 67.9%, while the malignant lesions constituted 24.5% with 104 cases as shown in Table 10 and Chart 2.

TABLE 11 DISTRIBUTION OF BENIGN AND MALIGNANT LESIONS OF BREAST

Breast Lesions (100 Cases)	**Benign Lesions**		**Malignant lesions**
	Non-Proliferative	**Proliferative**	
No. of cases	29	31	40
Percentage	29%	31%	40%

Out of the total 423 breast cytology cases 100 cases were randomly chosen taking into account 40 malignant and 60 benign cases encompassing both proliferative (31 cases) and non-proliferative lesions (29 cases) as shown in Table 11 and Chart 3. These 100 smears were

CHART 1 BREAST FNAC vs OTHER SITES

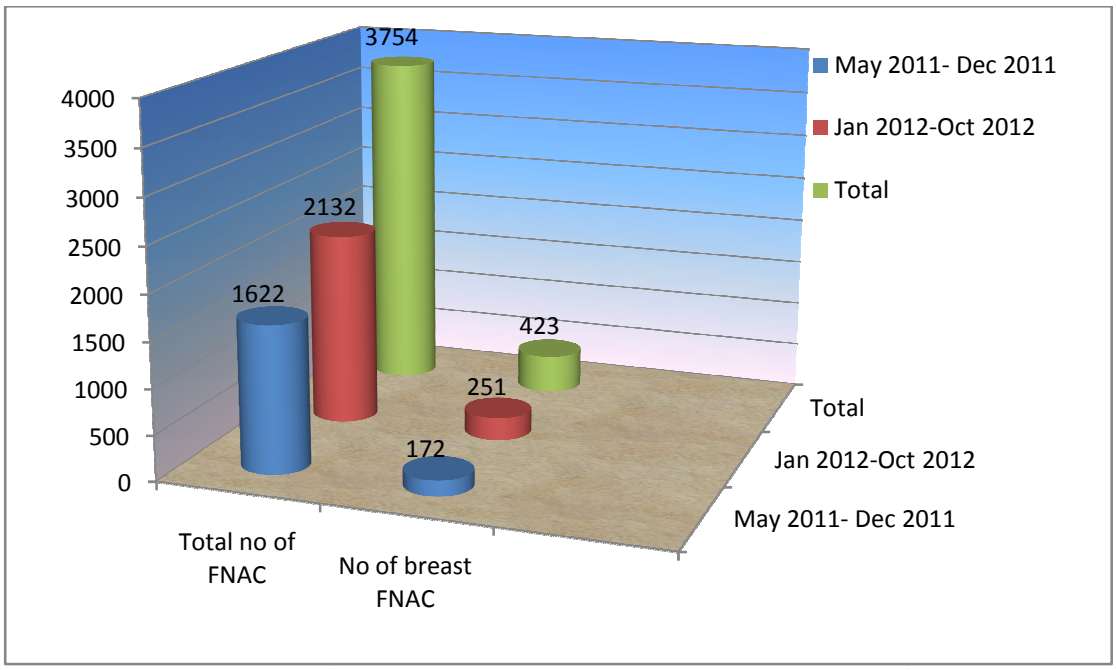
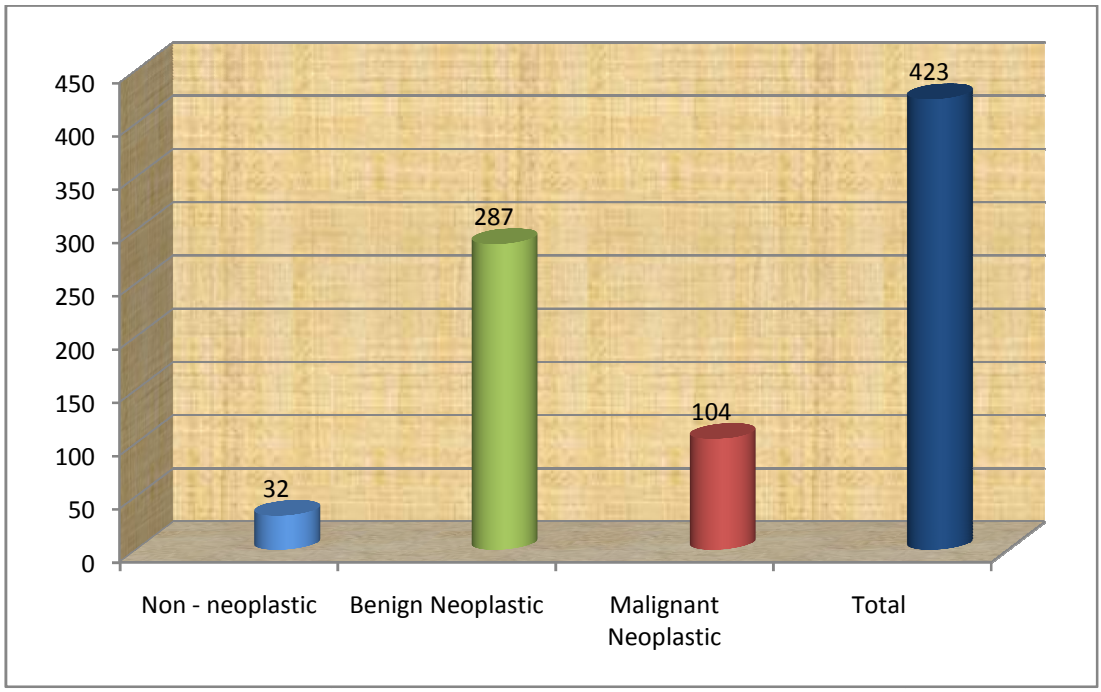


CHART 2 DISTRIBUTION OF NON-NEOPLASTIC AND NEOPLASTIC LESIONS ON BREAST CYTOLOGY



stained with AgNOR stain and examined meticulously for number of AgNOR dots and their morphology within the nucleus of the aspirated cells.

TABLE 12 DISTRIBUTION OF BENIGN LESIONS OF THE BREAST

Benign breast lesions (60 Cases)	Non-Proliferative lesions (Fibrocystic disease / Fibroadenosis)	Proliferative lesions		
		Epithelial Hyperplasia	Atypical Ductal Hyperplasia	Intra Ductal Papilloma
No. of cases	29	18	11	2
Percentage	48.33%	30%	18.33%	3.34%

Out of the 60 cases of benign lesions 29 cases constituting 48.33% fall in the group of non-proliferative lesions encompassing fibroadenosis / fibrocystic disease. The remaining 31 cases fall into the group of proliferative breast lesions with 18 cases of epithelial hyperplasia constituting 30%, 11 cases of atypical ductal hyperplasia constituting 18.33% and 2 cases of intra ductal papilloma constituting 3.34%. These findings are shown in Table 12.

TABLE 13 QUADRANT DISTRIBUTION OF BREAST LESIONS

Quadrant	Number of breast lesions	Percentage (%)
Upper outer	47	47%
Upper inner	12	12%
Lower outer	33	33%
Lower inner	6	6%
Central	2	2%

The palpable breast lesions were more common in the upper outer quadrant comprising 47%, the next to follow is lower outer quadrant comprising 33%, followed by upper inner quadrant (12%), lower inner quadrant (6%) and finally the central quadrant constituting the least number of cases with 2%. These findings were tabulated in Table13 and Chart 4.

TABLE 14 QUADRANT DISTRIBUTION OF BREAST CARCINOMAS

Quadrant	Number of breast Carcinomas	Percentage (%)
Upper outer	19	47.5%
Upper inner	5	12.5%
Lower outer	15	37.5%
Lower inner	1	2.5%

In our study breast carcinomas were most common in upper outer quadrant comprising 47.5% and the least common quadrant to be involved is the lower inner quadrant constituting 2.5%. The lower outer

CHART 3 DISTRIBUTION OF BENIGN AND MALIGNANT LESIONS OF BREAST

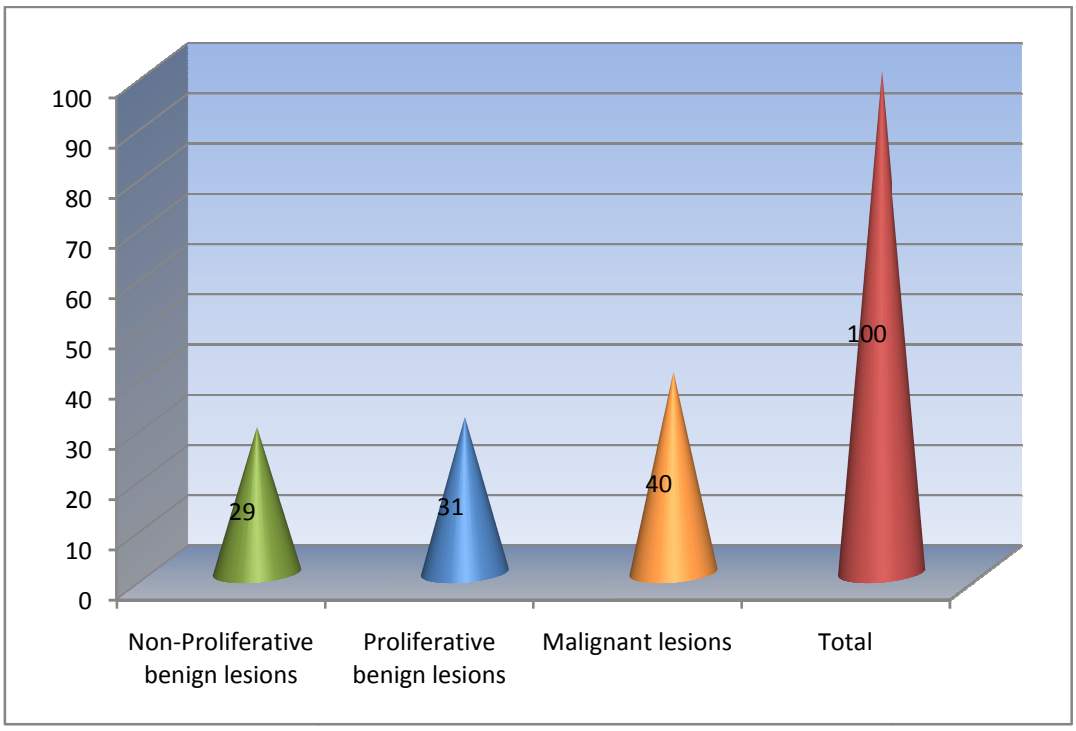
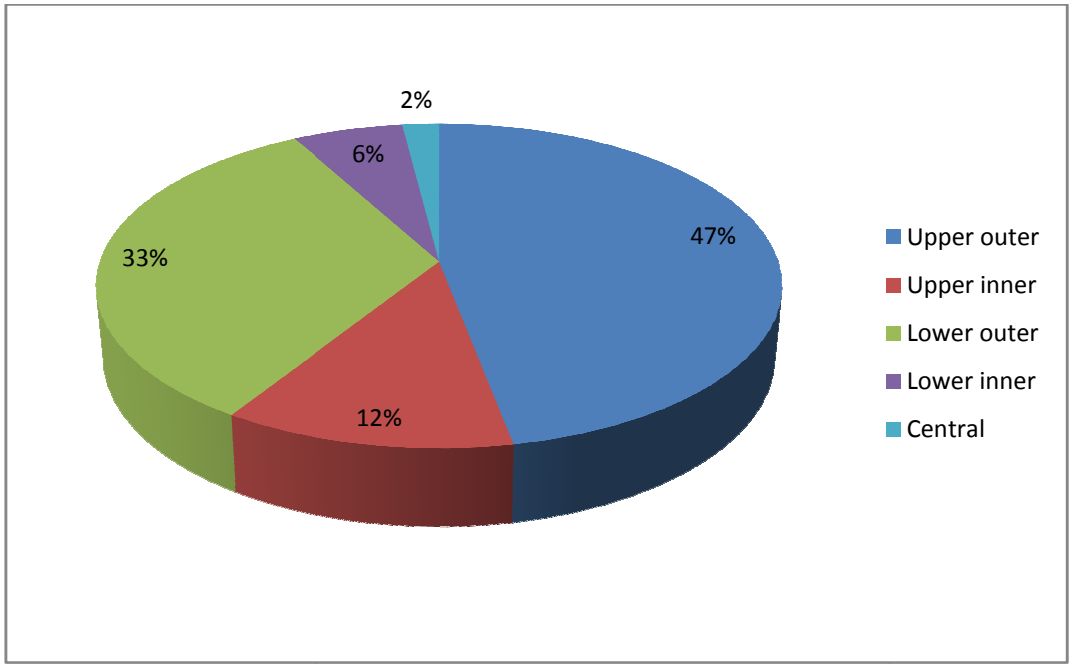


CHART 4 QUADRANT DISTRIBUTION OF BREAST LESIONS



quadrant constituting 37.5% and the upper inner quadrant constituting 12.5% were the second and the third most commonly involved quadrants respectively. These observations are shown in Table 14 and Chart 5.

TABLE 15 AGE DISTRIBUTION OF BREAST CARCINOMAS

Age groups	Number of breast carcinomas	Percentage (%)
30-39 yrs	3	7.5%
40-49 yrs	17	42.5%
50-59 yrs	12	30%
60-69 yrs	6	15%
70-79 yrs	2	5%
	Mean age = 49.5 yrs	Average age = 50.5yrs

By dividing the age group of patients into five categories we observed that breast carcinomas were more common in the age group of 40 – 49 years with 17 cases constituting 42.5%, followed by the age group of 50 – 59 years with 12 cases comprising 30 % and next to follow is the age group of 60 – 69 years with 6 cases constituting 15%.

The youngest patient in our study is 34 years old and the oldest patient in our study is 71 years of age. We arrived at a mean age of 49.5 years for occurrence of breast carcinoma in our study. These findings are tabulated in Table 15 and Chart 6.

CHART 5 QUADRANT DISTRIBUTION OF BREAST CARCINOMAS

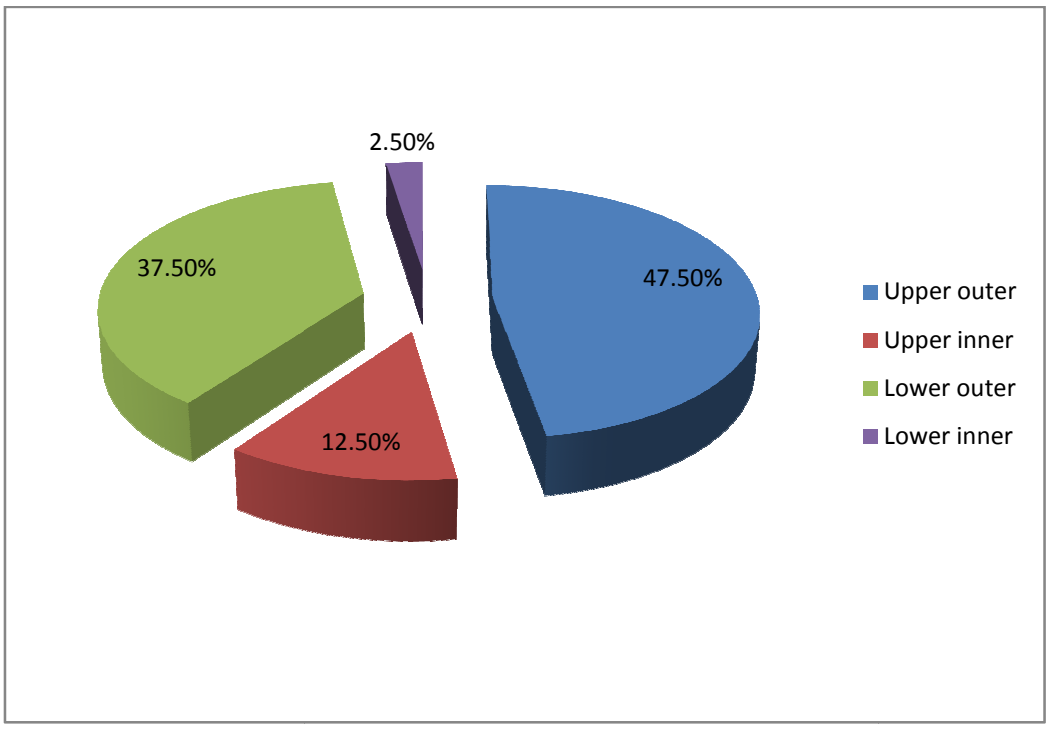


CHART 6 AGE DISTRIBUTION OF BREAST CARCINOMAS

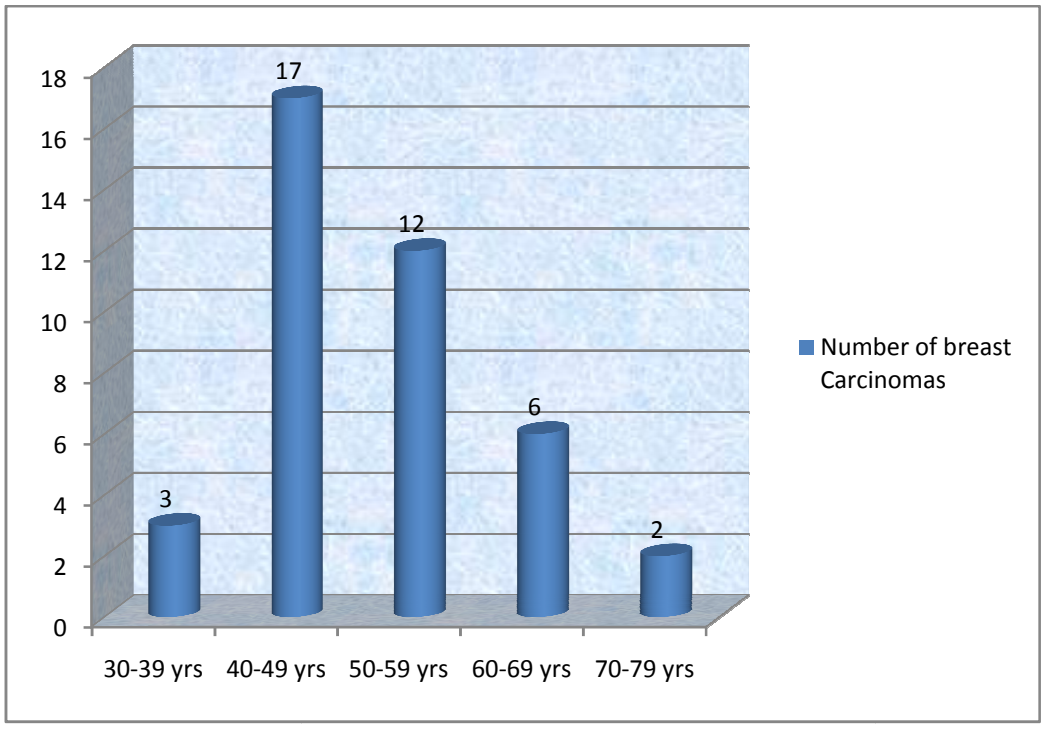


TABLE 16 CYTOLOGY GRADE DISTRIBUTION OF BREAST CARCINOMAS

Grade	Number of Cases	Percentage (%)
Grade I (6-11 score)	8	20%
Grade II (12-14 score)	26	65%
Grade III (15-18 score)	6	15%
Total	40	100%

All 40 cases of breast carcinomas were graded according to Robinson's grading system into three grades. Grade I carcinoma constituted 8 cases with a percentage of 20%, grade II carcinoma constituted the majority with 26 cases constituting 65% and grade III carcinoma constituted 6 cases with a percentage of 15%. These observations are shown in Table16 and Chart 7.

TABLE 17 CYTOLOGICAL GRADE vs TUMOUR SIZE

Size of tumour (cm)	Grade I (%)	Grade II (%)	Grade III (%)	Total	Percentage
<2 (T1)	2 (25%)	0	0	2	5%
2– 5 (T2)	6 (75%)	23 (88.5%)	5 (83.3%)	34	85%
>5 (T3)	0	3 (11.5%)	1 (16.7%)	4	10%
Total	8	26	6	40	100%

The association between cytological grade and tumour size was made in the Table 17 and Chart 8, which revealed most of the tumours (85%) to be in T2 (2 – 5cms) size. Among the Grade I tumours 75% (6

CHART 7 CYTOLOGY GRADE DISTRIBUTION OF BREAST CARCINOMAS

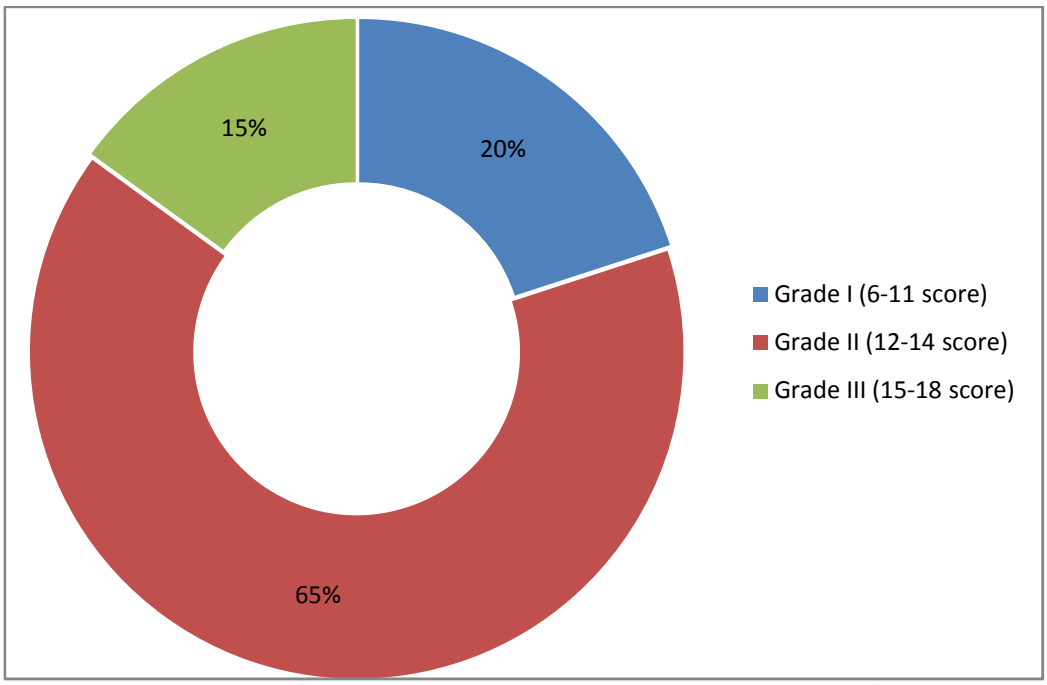
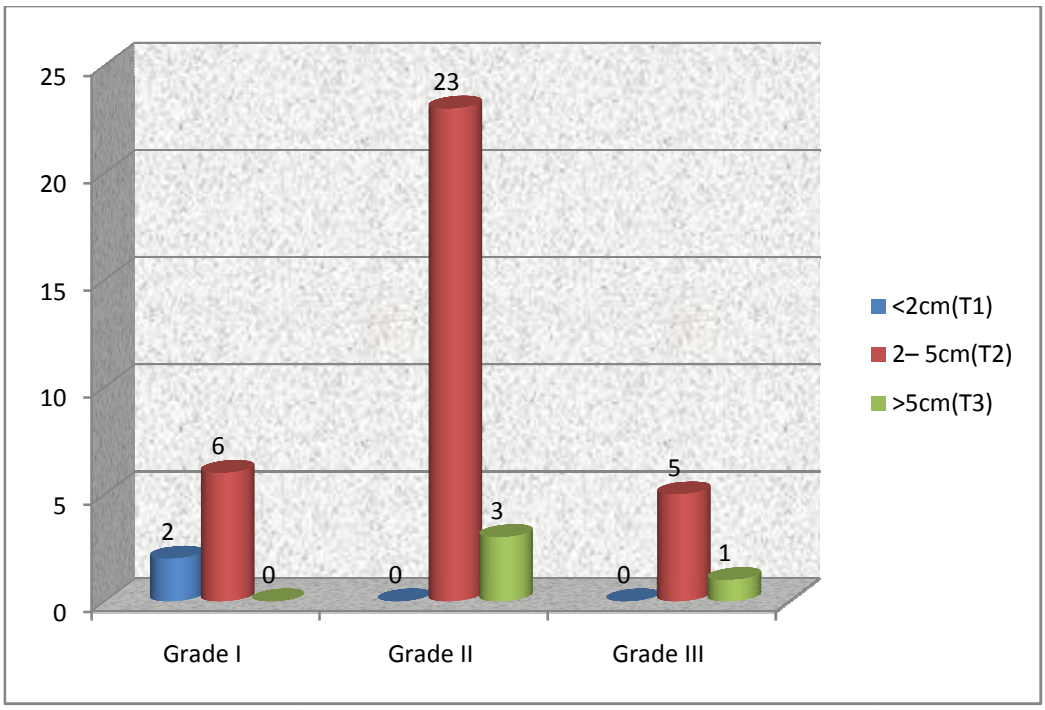


CHART 8 CYTOLOGICAL GRADE vs TUMOUR SIZE



cases) were in T2 and 25% (2 cases) were in T1. Among the Grade II tumours 88.5% (23 cases) were in T2 and 11.5% (3 cases) were in T3. Among the Grade III tumours 83.3% (5 cases) were in T2 and 16.7% (1case) was in T3 size. On applying the chi square test for statistical significance the P-value was ($0.05 \leq 0.05$ – sig). Hence the correlation between cytological grade and tumour size was significant.

TABLE18 CYTOLOGICAL GRADE vs PATIENT AGE

Patient age	Grade I (%)	Grade II (%)	Grade III (%)	Total	Percentage
30-39 yrs	0	3 (11.5%)	0	3	7.5%
40-49 yrs	4 (50%)	12 (46.2%)	1 (16.7%)	17	42.5%
50-59 yrs	2 (25%)	7 (26.9%)	3 (50%)	12	30%
60-69 yrs	1 (12.5%)	3 (11.5%)	2 (33.3%)	6	15%
70-79 yrs	1 (12.5%)	1 (3.9%)	0	2	5%
Total	8	26	6	40	

The Table 18 and Chart 9 depicts that grade III tumours were more common in the age group of 50 to 59 years (50%) followed by the age group of 60 to 69 years constituting 33.3%. The age group of 40 to 49 years is the most common age group affected in both grade I (46.2%) and grade II (50%) tumours. There was no statistical correlation between patient age and tumour grade (P-value – $0.586 > 0.05$ – Not sig).

TABLE19 CYTOLOGICAL GRADE vs LYMPH NODE STATUS

Lymph node status	Grade I (%)	Grade II (%)	Grade III (%)	Total	Percentage
Positive	1 (12.5%)	11 (42.3%)	5 (83.3%)	17	42.5%
Negative	7 (87.5%)	15 (57.7%)	1 (16.7%)	23	57.5%
Total	8	26	6	40	100%

Among the grade I tumours 87.5% showed negative lymph node status and only 12.5% showed positive lymph nodes. On the other hand grade III tumours showed 83.3% positive and 16.7% negative lymph node status. Grade II tumours showed equivocal findings with 42.3% showing lymph node positivity and 57.7% showing lymph node negativity. These findings are depicted in Table 19 and Chart 10. There was a significant statistical correlation between grade of the tumour and lymph node status (P-value – 0.030 < 0.05 – sig).

CHART 9 CYTOLOGICAL GRADE vs PATIENT AGE

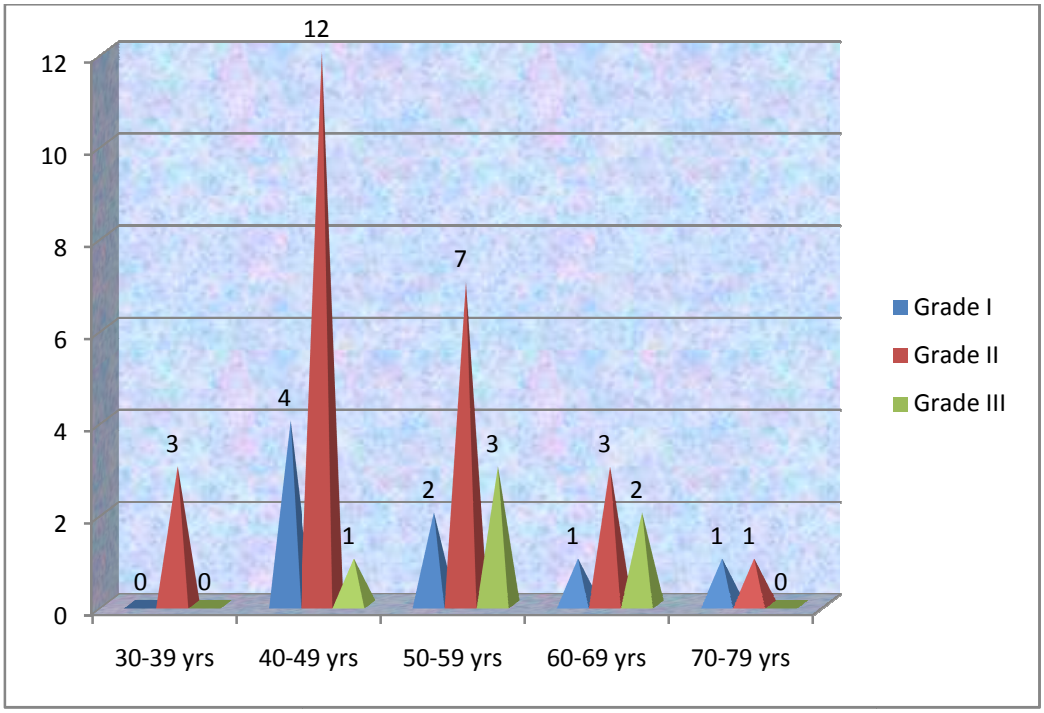


CHART 10 CYTOLOGICAL GRADE vs LYMPH NODE STATUS

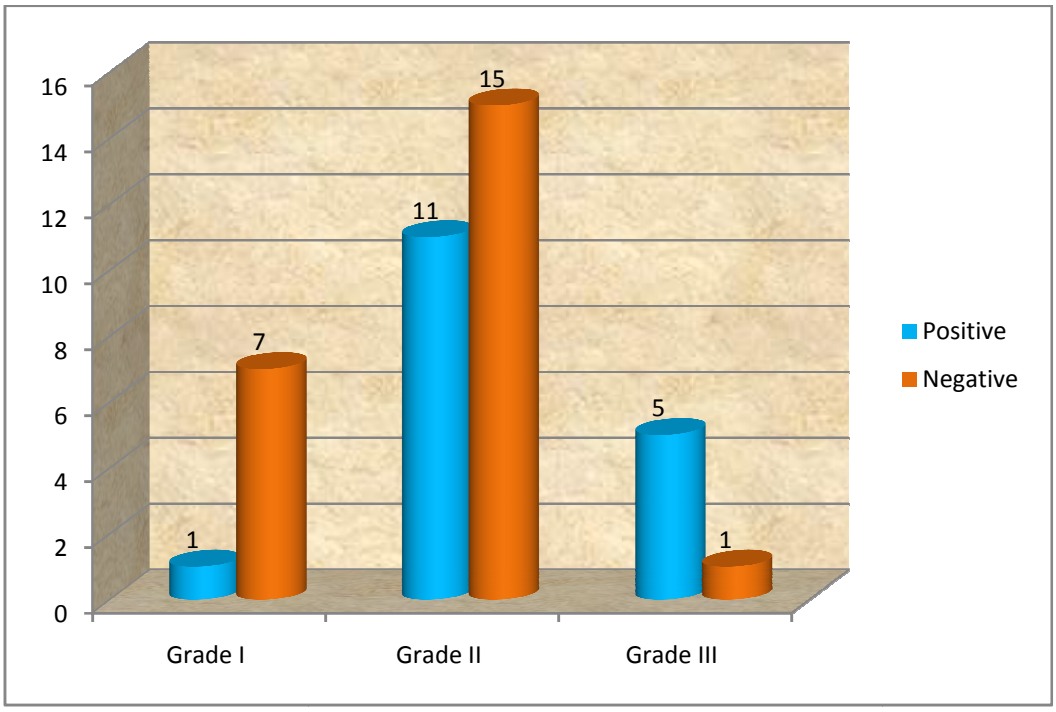


TABLE 20 AgNOR SCORE OF BENIGN vs MALIGNANT BREAST LESIONS

AgNOR parameters	Benign lesions			Malignant lesions			P-value
	Range	Mean	S.D.	Range	Mean	S.D.	
Mean AgNOR	1.67-6.21	3.55	+/-1.323	5.8-9.54	7.352	+/-0.822	<0.001*
p AgNOR	3 - 51	23.6	+/-13.059	40 - 71	50.875	+/-6.603	<0.001*
AgNOR size variation	0 - 2	0.7	+/-0.646	2 – 3	2.25	+/-0.439	<0.001*
AgNOR Distribution	0 - 2	0.717	+/-0.613	2 – 3	2.25	+/-0.439	<0.001*
SAPA score	3 - 5	3.717	+/-0.783	5 – 8	6.7	+/-0.883	<0.001*

The AgNOR staining and enumeration was done on 100 cases which revealed mean AgNOR score of 3.554+/-1.323 in benign lesions, while mean AgNOR was higher in malignant lesions with a score of 7.352+/-0.822. The proliferative AgNOR index also showed higher values in malignant lesions (50.875) compared to benign lesions (23.6). AgNOR size variation and distribution within nucleus also showed higher values in malignant lesions compared to their benign counterparts. Mean SAPA score was 6.7 in malignant lesions compared to 3.717 in benign breast lesions. All the AgNOR parameters showed a statistically significant higher values in malignant lesions compared to the benign lesions (P value – 0. 001* < 0.05 – sig). These findings are tabulated in Table 20 and Chart 11 (* - Highly significant value).

TABLE 21 AgNOR SCORE OF NON-PROLIFERATIVE vs PROLIFERATIVE BREAST LESIONS

AgNOR parameters	Non-proliferative			Proliferative lesions			P-value
	Range	Mean	S.D.	Range	Mean	S.D.	
Mean AgNOR	1.67-3.35	2.382	+/- 0.546	2.78-6.21	4.651	+/- 0.772	<0.001*
p AgNOR	3 - 25	12.414	+/- 6.417	18 – 51	30.065	+/- 7.929	<0.001*
AgNOR size variation	0 - 1	0.172	+/- 0.384	1 – 2	1.194	+/- 0.402	<0.001*
AgNOR Distribution	0 - 1	0.241	+/- 0.435	1 – 2	1.161	+/- 0.374	<0.001*
SAPA score	3 - 4	3.034	+/- 0.186	3 – 5	4.355	+/- 0.551	<0.001*

Out of 60 benign lesions 29 were non-proliferative and 31 were proliferative lesions. The proliferative lesions encompassing epithelial hyperplasia, intra ductal papilloma and atypical ductal hyperplasia showed significantly higher AgNOR values in terms of mean AgNOR, proliferative AgNOR index, SAPA score, AgNOR size variation and distribution within nucleus, compared to the non-proliferative benign lesions encompassing fibrocystic disease / fibroadenosis (Fig. 3-6). These AgNOR value showed a statistically significant variation between proliferative and non-proliferative lesions of the breast (P-value – 0. 001* < 0.05 – sig). These values are shown in Table 21 and Chart 12.

CHART 11 AGNOR SCORE OF BENIGN VS MALIGNANT BREAST LESIONS

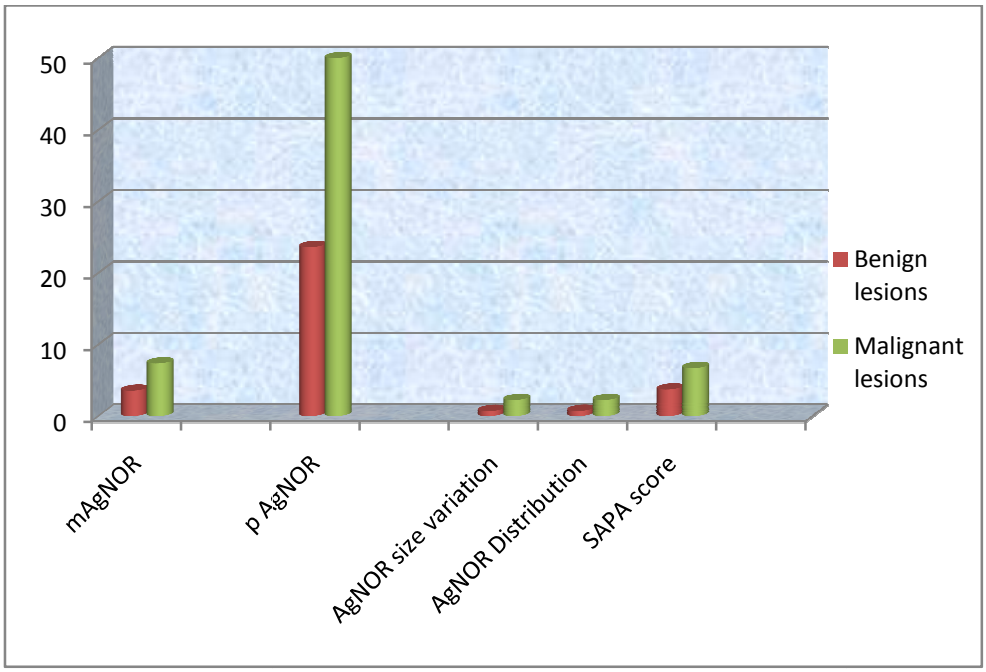


CHART 12 AGNOR SCORE OF NON-PROLIFERATIVE VS PROLIFERATIVE BREAST LESIONS

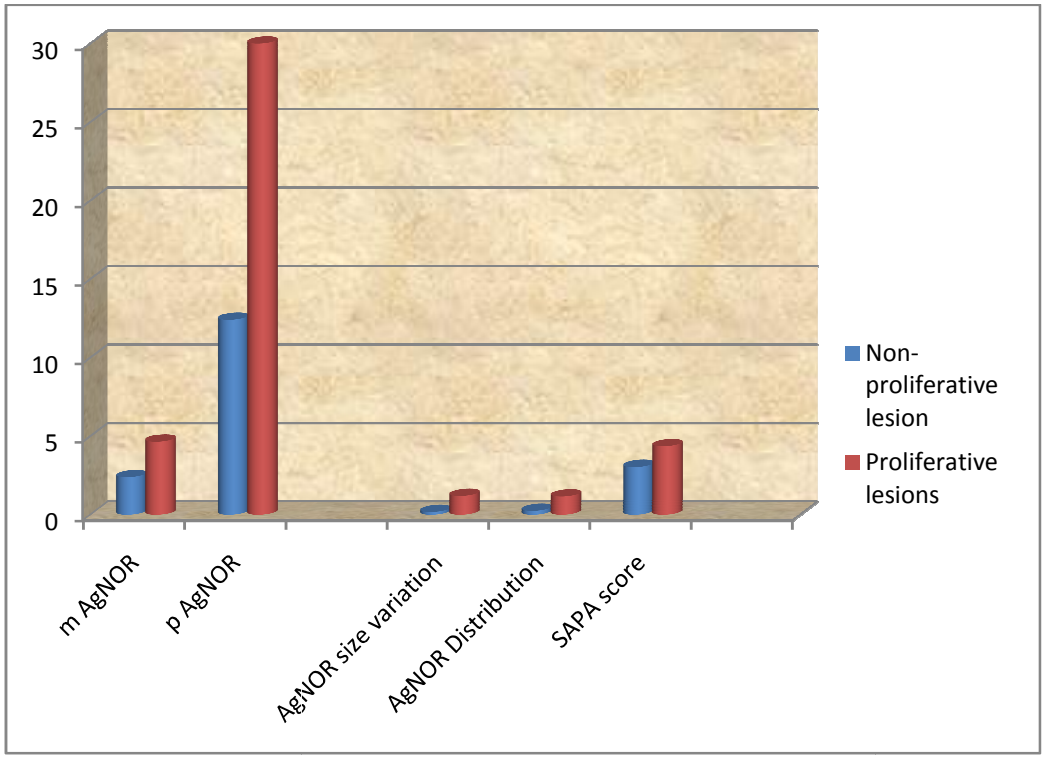


TABLE 22 AgNOR SCORE OF PROLIFERATIVE EPITHELIAL BREAST LESIONS

AgNOR parameters	Epithelial Hyperplasia		Atypical Ductal Hyperplasia		Intra Ductal Papilloma		P-value
	Mean	S.D.	Mean	S.D.	Mean	S.D.	
Mean AgNOR	4.419	+/- 0.843	4.903	+/- 0.519	5.345	+/- 0.686	<0.001*
p AgNOR	31.72	+/- 8.498	36.727	+/- 6.278	40.5	+/- 2.121	<0.001*
AgNOR size variation	1.167	+/- 0.383	1.182	+/- 0.405	1.5	+/- 0.707	0.021<0.05**
AgNOR Distribution	1.167	+/- 0.383	1.182	+/- 0.405	1	0	0.052
SAPA score	4.333	+/- 0.485	4.364	+/- 0.674	4.5	+/- 0.707	0.542

Out of the 31 benign proliferative epithelial breast lesions, there was a gradual and steady increase in AgNOR values from epithelial hyperplasia (Fig. 7-10) to atypical ductal hyperplasia (Fig. 11-14) and then to intra ductal papilloma (Fig.15-18). But the differences were very subtle and there was overlap between the AgNOR counts with regard to individual values of these lesions. Only the mAgNOR and pAgNOR values were found to be statistically significant in differentiating various subtypes of benign proliferative epithelial breast lesions (P-value – 0. 001* < 0.05 – sig). These values are tabulated in Table 22.

TABLE 23 ROBINSON'S SCORE vs mAgNOR SCORE

No of cases	Robinson Grade	Robinson's Score			Mean AgNOR			P-value
		Range	Mean	S.D.	Range	Mean	S.D.	
8	I	7-10	8.875	+/- 1.125	5.8-7.36	6.569	+/- 0.648	<0.001*
26	II	12-14	12.96	+/- 0.774	6–8.71	7.37	+/- 0.656	<0.001*
6	III	15-17	16.17	+/- 0.753	7.87-9.84	8.322	+/- 0.647	<0.001*

Correlation between the Robinson's score and AgNOR score in the 40 cases of malignant breast neoplasm is tabulated in Table 23. The mean AgNOR score showed a gradual increase in correlation with the Robinson's score and thus with the grade of the carcinomatous lesion. The mean AgNOR score and the Robinson's score showed a statistically significant correlation with regard to malignant neoplasm of breast (P-value – 0. 001* < 0.05 – sig).

TABLE 24 ROBINSON'S SCORE vs pAgNOR SCORE

No of cases	Robinson Grade	Robinson's Score			pAgNOR			P-value
		Range	Mean	S.D.	Range	Mean	S.D.	
8	I	7 - 10	8.875	+/- 1.125	40 - 52	44.5	+/- 4.472	<0.001*
26	II	12-14	12.96	+/- 0.774	41 - 62	51.12	+/- 4.893	0.141
6	III	15-17	16.167	+/- 0.753	49 - 71	58.34	+/- 7.763	<0.001*

As with the mean AgNOR score the proliferative AgNOR index also showed a good correlation with that of Robinson's score. The proliferative AgNOR index score increased with increasing grades of the Robinson's grading system for breast carcinomas. But these were significant statistically only for grade I (Fig. 19-22) and grade III tumours (Fig. 27-30) (P-value – 0. 001* < 0.05 – sig) and not for grade II tumours (Fig. 23-26) (P-value – 0.141 > 0.05 – Not sig). The findings are depicted in Table 24.

TABLE 25 ROBINSON'S SCORE vs AgNOR SIZE VARIATION

No of cases	Robinson Grade	Robinson's Score			AgNOR size variation			P-value
		Range	Mean	S.D.	Range	Mean	S.D.	
8	I	7-10	8.875	+/- 1.125	2 - 2	2	+/- 0	<0.001*
26	II	12-14	12.96	+/- 0.774	2-3	2.154	+/- 0.368	<0.001*
6	III	15-17	16.167	+/- 0.753	3 - 3	3	+/- 0	<0.001*

There was an increased variation in the size of the AgNOR dots as the Robinson's cytology grade of the malignant breast neoplasm increased. Thus AgNOR dot size variation correlated very well with that of Robinson's scoring system and is of statistical significance (P-value – 0. 001* < 0.05 – sig). These findings were depicted in Table 25.

TABLE 26 ROBINSON'S SCORE vs AgNOR DISTRIBUTION IN NUCLEI

No of cases	Robinson Grade	Robinson's Score			AgNOR distribution in nuclei			P-value
		Range	Mean	S.D.	Range	Mean	S.D.	
8	I	7 - 10	8.875	+/- 1.125	2 - 2	2	+/- 0	<0.001*
26	II	12 -14	12.96	+/- 0.774	2-3	2.154	+/- 0.368	<0.001*
6	III	15-17	16.167	+/- 0.753	3 - 3	3	+/- 0	<0.001*

There was an increased dispersion of AgNOR dots within the nucleus as the cytology grade of the malignant neoplasm increased. Thus there is a statistically significant correlation between AgNOR dot distribution and Robinson's cytology score (P value – 0. 001* < 0.05–sig) as shown in Table 26.

TABLE 27 ROBINSON'S SCORE vs SAPA SCORE

No of cases	Robinson Grade	Robinson's Score			SAPA score			P-value
		Range	Mean	S.D.	Range	Mean	S.D.	
8	I	7 - 10	8.875	+/- 1.125	5 - 7	5.75	+/- 0.707	<0.001*
26	II	12-14	12.96	+/- 0.774	6 - 8	6.731	+/- 0.368	<0.001*
6	III	15-17	16.167	+/- 0.753	7 - 8	7.833	+/- 0.378	<0.001*

The SAPA score of the malignant breast neoplasm increased as the grade and thus the aggressiveness of the neoplasm increased. Thus SAPA score and Robinson's cytology score goes hand in hand with each other. The SAPA score and the Robinson's cytology score showed a statistically significant correlation with regard to malignant neoplasm of breast (P-value – 0. 001* < 0.05 – sig). These findings are shown in Table 27.

TABLE 28 AgNOR SCORE vs LYMPH NODE STATUS

AgNOR parameters	Negative lymph nodes		Positive lymph nodes	
	Mean	S.D.	Mean	S.D.
Mean AgNOR	7.045	+/-0.763	7.768	+/-0.726
p AgNOR	49.565	+/-6.57	52.647	+/-6.412
AgNOR size variation	2.13	+/-0.344	2.412	+/-0.507
AgNOR Distribution	2.13	+/-0.344	2.412	+/-0.507
SAPA score	6.391	+/-0.783	7.117	+/-0.857

The patients with positive lymph node status show a mild increase in AgNOR values compared to that of node negative patients. The AgNOR score is not of much significance in delineating these patients according to lymph node status. All the parameters of AgNOR values with regard to lymph node status are shown in Table 28.

TABLE 29 ROBINSON'S SCORE vs LYMPH NODE STATUS

Lymph node status	Robinson's Score	
	Mean	S.D.
Negative lymph nodes	11.826	+/-2.289
Positive lymph nodes	13.706	+/-2.054

The Robinson's score also showed not much of a significant variation in the values with regard to the axillary lymph node status of the patients, these findings were tabulated in Table 29.

TABLE 30 AgNOR SCORE vs TUMOUR SIZE

AgNOR parameters	< 2 cms (T1)		2 – 5 cms (T2)		> 5 cms (T3)		P-value
	Mean	S.D.	Mean	S.D.	Mean	S.D.	
Mean AgNOR	6	+/- 0.198	7.332	+/- 0.644	8.2	+/-1.427	<0.001*
p AgNOR	41.5	+/- 2.121	50.912	+/- 5.160	55.25	+/-13.72	<0.001*
AgNOR size variation	2	0	2.206	+/- 0.410	2.75	+/-0.5	<0.001*
AgNOR Distribution	2	0	2.206	+/- 0.410	2.75	+/-0.5	<0.001*
SAPA score	6	0	6.765	+/- 0.699	7.5	+/-1.00	<0.001*

There was a good correlation between the AgNOR values and the tumour size. The AgNOR counts increased gradually as the size of the malignant neoplasm increased, with T1 lesions showing a mAgNOR count of 6, T2 lesions showing a mAgNOR count of 7.332 and T3 lesions showing the highest mean AgNOR count of 8.2. The AgNOR score and the size of the breast neoplasm showed a statistically significant correlation between them (P-value – 0. 001* < 0.05 – sig). These observations were depicted in Table 30.

TABLE 31 ROBINSON'S SCORE vs TUMOUR SIZE

Tumour Size	Robinson's Score		P-value
	Mean	S.D.	
< 2 cms (T1)	7.5	+/-0.707	<0.001*
2 – 5 cms (T2)	12.735	+/-2.064	
> 5 cms (T3)	14.25	+/-2.062	

The Robinson's score also showed a gradual increase in the values as the size of tumour increased with T1 lesions having a mean value of 7.5, T2 lesion showing a mean value of 12.735 and finally T3 lesion had the highest mean value of 14.25. Hence there is a statistically significant correlation between Robinson's score and the size of the breast neoplasm (P-value – 0. 001* < 0.05 – sig). These observations were shown in Table 31.

Out of the 40 breast malignancies detected by cytology all were confirmed by histopathological examination. 5 cases were found to be of grade I tumours, 20 were of grade II tumours and 15 were of grade III tumours according to Bloom and Richardson histological grading system

Among the 8 breast carcinoma cases detected by cytology as Robinson's grade I lesions 5 turned out to be of grade I in histology while remaining 3 turned out to be grade II tumours in histology. Out of the 26 cases detected to be grade II lesions in cytology 17 turned out to be of the same grade in histology while the remaining 9 cases belonged to grade III tumours in histology. Of the 6 cases detected to be grade III lesions in cytology all turned out to be of the same grade III in histology.

DISCUSSION

The initial step in the evaluation of any patient presenting with a palpable breast lesion is to distinguish benign lesions from malignant ones. Fine Needle Aspiration Cytology (FNAC) being a simple, rapid, less invasive and cost effective preoperative out-patient procedure plays an immense role in the management of these types of lesions. It can also provide additional information relevant for diagnosis and treatment[38]. But FNAC does pose an increasing challenge and diagnostic dilemmas in certain situations. This can to a certain extent be overcome by using an ancillary cell proliferative marker in the form of AgNOR stain which is a simple, one step silver staining technique.

AgNORs can be easily demonstrated in routinely fixed cytology smears and can even be applied on already H & E stained smears after de-staining them. The frequency, number and dispersion of AgNOR dots are consistently higher in malignant neoplasm compared to the benign neoplasm of breast. In this regard AgNORs have been attracting much attention as a proliferative marker and as an indicator for aggressiveness of the lesion and has a potential value in diagnostic cytology[52].

Robinson's cytological method of grading was found to be more specific compared to other cytological grading methods when Bloom and

Richardson histological grading system is taken as a gold standard in the study conducted by Das et al[35].

Our present study was conducted on 100 cases of breast lesions encompassing both benign (proliferative and non-proliferative) and malignant lesions. The cytology smears were stained with both Hematoxylin & Eosin and AgNOR stains. The AgNOR dot number and morphology is enumerated meticulously in each case and the findings tabulated. Similarly Robinson's score was also calculated for each individual case and findings tabulated. The current study evaluates the significance of AgNOR score in distinguishing benign from malignant neoplasm of breast, and also in differentiating non-proliferative from proliferative breast lesions. The study also evaluates the significance of AgNOR score in relation to Robinson's score in the grading of malignant breast lesions.

Our study includes 100 cases of breast lesions of which 40 cases were malignant and 60 cases were benign lesions. Among the benign lesions 29 cases were non-proliferative breast diseases encompassing fibroadenosis / fibrocystic disease, the remaining 31 cases were proliferative breast lesions including 11 cases of atypical ductal hyperplasia, 18 cases of epithelial hyperplasia and 2 cases of intra ductal papilloma.

In the studies conducted by Mi-Jung Kim et al in 2006 showed a mean age of 47.4 years for breast carcinomas, Gloria Piero et al. in the same year showed a mean age of 54 years and Aye Aye Thike et al. in 2010 showed a mean age of 53 years for patients presenting with breast carcinomas. In our present study age of the patients ranged from 17 years to 71 years. Among the malignant lesions the youngest patient was of 34 years and the oldest patient was of age 71 years. Among the benign lesions the youngest patient was of age 17 years and the oldest patient was of age 69 years. Among 40 cases of malignant lesions the mean age of the patient was 49.5 years, and the mean age group was 40 – 49 years, which correlated with the above studies conducted by Mi-Jung Kim et al, Gloria Piero et al and Aye Aye Thike et al.

Studies conducted by Azzopardi[20] and Weidner[68] showed upper outer quadrant to be the most common quadrant involved by breast carcinoma, the least common quadrant to be involved was the lower inner quadrant. Our present study correlated with the findings of the above studies showing 47 % of the breast lesions to be localised to upper outer quadrant followed by lower outer quadrant having 33% of cases, the least common quadrant affected in our study was central quadrant accounting 2%. Among the malignant breast lesions the most common quadrant affected was upper outer accounting for 47.5% followed by lower outer

accounting for 37.5%, the least common quadrant was lower inner constituting 2.5%.

Study conducted by Lobna Ayadhi et al[69] showed T2 size lesions forming the majority (63.2%) of the breast carcinomas, followed by T3 and T1 sized lesions. Kakil Rassul et al[70] also showed similar findings with T2 lesions being most common followed by T3 and T1 lesions. While Lakmini et al[71] showed T3 lesions being more common followed by T2 and T1. Our present study showed a higher percentage of tumours to be in the T2 size group (85%), followed by T3 lesions showing 10% and T1 lesions showing 5%. Our findings were similar to the studies conducted by Lobna Ayadhi et al and Kakil Rassul et al, while contradicts the study conducted by Lakmini et al.

Studies carried out by Carey et al[72] and Madhuri et al[73] showed grade III tumours of the breast to be the most common constituting 49% followed by grade II tumours. While our present study showed the grade II tumours of the breast to be more frequent than other grades constituting 65% followed by grade I and grade III tumours which constituted 20% and 15% respectively. Our findings differed from the above mentioned studies.

AgNOR – BENIGN LESIONS:

Simba M et al[62] had a mean AgNOR count of 1.8 for benign lesions of the breast. Dasgupta A et al[74] also reported similar mean AgNOR value of 1.61 for benign breast lesions and Kumar A et al[63] demonstrated the mean AgNOR count of 1.88 in the benign breast lesions. Whereas Reddy GS et al.[59] reported a higher mAgNOR count of 7.45 and Drevan PA et al.[65] had a wide range of values for mAgNOR of benign lesions of breast ranging from 2.65 to 6.8 similar to that of our study. In our present study the mean AgNOR values were consistently higher in malignant neoplasm compared to the benign neoplasm and were also statistically significant. The mean AgNOR count of benign neoplasm (60 cases) was 3.55+/-1.323, with wide range of values ranging from 1.67 to 6.21 and a SAPA score of 3.72.

Among the 60 cases of benign lesions 29 cases of non-proliferative breast lesions showed a mean AgNOR count of 2.38 with values ranging from 1.67 to 3.35 and a SAPA score of 3.03, the remaining 31cases of proliferative breast lesions showed a higher mean AgNOR score of 4.651 with values ranging from 2.78 to 6.21 and a SAPA score of 4.35. The mean AgNOR, proliferative AgNOR index, AgNOR size variation, pattern of AgNOR distribution within nucleus and SAPA score all

showed a higher AgNOR values in proliferative lesions compared to non-proliferative lesions of breast.

Among the 31 cases of proliferative breast lesions 18 cases of epithelial hyperplasia showed a mean AgNOR value of 4.419+/-0.843 and a SAPA score of 4.333+/-0.485, 11 cases of atypical ductal hyperplasia showed mAgNOR value of 4.903+/-0.519 and a SAPA score of 4.364+/-0.674 and 2 cases of intraductal papilloma showed mAgNOR value of 5.345+/-0.686 and a SAPA score of 4.5+/-0.707. The variation in AgNOR values between the different subtypes of proliferative breast lesions is very subtle. The mAgNOR and pAgNOR values were only found to be statistically significant in differentiating subtypes of benign proliferative epithelial breast lesions on cytology (P-value – 0. 001* < 0.05 – sig). While AgNOR size variation, pattern of AgNOR distribution within nucleus and SAPA score are not found to be of much significance. There are a few overlaps between AgNOR score with regard to individual values of different subtypes.

AgNOR – MALIGNANT LESIONS:

Kim A[75] reported a mean AgNOR count for malignant breast lesions as 5.09, Simba M et al[62] had a lower mean AgNOR count of 3.5 for malignant breast lesions compared to our study, while Kumar A et al[63] reported a mAgNOR count of 6.57 for malignant lesions which was

similar to our study. Whereas Dasgupta A et al[74] reported a higher mAgNOR value of 12.10 for malignant breast lesions, similar to that of Reddy GS et al[59] who reported a value of 12.72 for breast malignancies. Drevan PA et al[65] reported a wide range of values for mAgNOR from 4.6 to 26.9 with respect to malignant breast lesions.

In our present study malignant breast neoplasm showed a mAgNOR value of 7.352+/-0.822 with individual values ranging from 5.8 to 9.54 and with a SAPA score of 6.7. Among 40 malignant lesions 8 cases of grade I tumours showed a mAgNOR value of 6.569+/-0.648 with a SAPA score of 5.75, grade II tumours showed a mAgNOR value of 7.37+/-0.656 with a SAPA score of 6.73 and grade III tumours showed a mAgNOR value of 8.322+/-0.647 with a SAPA score of 7.83. There was a steady increase in mAgNOR values and SAPA score in concordance with the grade of the lesions. The mAgNOR values showed a statistically significant correlation with the Robinson's cytological grade. All the other AgNOR parameters also showed a gradual and steady increase in the values as the Robinson's cytological grade of the tumour progressed higher and was found to be statistically significant.

MALIGNANT VS BENIGN LESIONS:

Simba M et al[62], who studied the cytology of 200 breast cases including 140 malignancies, 55 benign lesions and 5 normal breasts came

out with the finding that AgNOR values are much higher in malignant neoplasm compared to the benign ones. The study conducted by Dasgupta A et al[74] showed AgNOR values are of not much significance in differentiating between two subtypes of benign lesion (fibroadenoma and fibrocystic disease) which was in concordance with our study. He also showed higher AgNOR values for malignancies compared to benign lesions.

Roller E et al.[58] had similar findings with higher AgNOR counts for malignant neoplasm of breast compared to benign neoplasm. Reddy GS, Sesikeran B, Bhaskaran CS[59] conducted study on 10 benign and malignant epithelial lesions of breast and found higher AgNOR values for malignant lesions compared to benign lesions. Hasnan J, Jayaram G[40] conducted a study on 31 cases of benign lesions and 25 cases of malignant lesions of breast and made observations that AgNOR value in benign lesions ranged from 2.55 to 5.0 in contrast with malignant lesions which showed values ranging from 5.8 to 17.2, they also observed that there was no overlap between the AgNOR values of benign and malignant lesions of breast.

Meehan SM, Carney DN, Magee H, Dervan PA[54] conducted a study on the value of AgNOR in differentiating malignant and benign breast lesions in cytology. They observed a mean AgNOR value of 4.44

for benign breast lesions and 9.52 for malignant breast lesions. They arrived at a median AgNOR score of 7 for benign lesions and 13 for malignant lesions. They put forth a diagnostic accuracy of 90% for AgNORs in differentiating benign from malignant lesions.

Khanna AK, Kumar M, Ansari MA, Khanna A[64] assessed 27 benign and 46 malignant breast lesions. They used two parameters both Subjective AgNOR Pattern Assessment (SAPA) score and mean AgNOR count. They found that mAgNOR score and SAPA were quite similar in differentiating benign from malignant lesions. The mean AgNOR score of benign lesion was 2.75 compared to malignant lesion which showed a value of 6.94. These findings are similar to that observed in our study. This study showed a SAPA score of 5.87 for benign lesions compared to 9.02 for malignant lesions. Similarly Kumar A, Kumar M, Kushwaha AK, Gupta S[63] also had higher AgNOR values for malignant lesions compared to benign lesions of breast.

Karmakar T, Radhika S, Gupta SK[60] found a higher mean AgNOR value of 16.63 for malignant lesion and mean AgNOR value of 6.39 for benign lesions. The overall AgNOR values are higher compared to our study. They concluded by putting forth a cut-off value of 11 for differentiating benign from malignant lesions. Mehrotra A, Chandra T[61] assessed the cytological smears of 64 malignant and 31 benign neoplasm

of breast and concluded by stating that the cut off point of 4 can be used in differentiating benign from malignant breast lesions with regard to mean AgNOR counts.

Our present study showed a statistically significant difference between the AgNOR values of benign lesions compared to that of malignant lesions of breast. Our study showed a mAgNOR value of 3.554+/-1.323 for benign lesions with individual values ranging from 1.67 to 6.21. While the malignant lesions showed a mAgNOR value of 7.352+/-0.822 with individual values ranging from 5.8 to 9.54. Our study showed mean pAgNOR value of 23.6+/-13.059 for benign lesions compared to the mean pAgNOR value of 50.875+/-6.603 for malignant lesions of breast. Our present study showed a considerable variation in the AgNOR size and distribution within nucleus for malignant neoplasm compared to a meagre variation in the AgNOR size and distribution for the benign neoplasm of breast. Our study also showed a higher mean SAPA score of 6.7+/-0.883 for malignant neoplasm compared to lower mean SAPA score of 3.717+/-0.783 for benign neoplasm of breast.

In a study by Dhakhwa R et al[55] on 110 breast lumps they observed that if the cut off score for AgNOR count / nucleus is taken as 6 specificity is 88.9%, sensitivity is 89.5% and if the cut off value for SAPA score is taken as 8 specificity is 83.3%, sensitivity is 89.5% for

differentiating benign from malignant neoplasms. From our study we observed that a mAgNOR cut off value of 6 and SAPA score cut off value of 5 can be effectively utilised in differentiating benign from malignant neoplasm of the breast with a sensitivity of 92.5% and specificity of 90%.

Ruschoff J, Plate K, Contractor H, Neumann K, Thomas C[76] found a considerable overlap of mean AgNOR score between malignant and benign lesions. They found the mAgNOR values for benign lesions in the range of 1.2 to 3.8 and the mAgNOR values for malignant lesions in the range of 1.5 to 16.2. Giri DD, Dundas SA, Lawry J, Nottingham JF, Underwood JC[77] also noted overlapping of AgNOR counts in 25 to 30% of carcinomas with epithelial hyperplastic lesions in the range of 2 to 3 AgNOR dots per nuclear profile. In our present study 3 of the cases, 2 from epithelial hyperplasia and 1 from atypical ductal hyperplasia showed a mild overlap in the mAgNOR count with that of malignant lesions of breast.

There was a considerable variation in the absolute value of mean AgNOR counts by different studies. These can be attributed to the fact that different authors count AgNOR dots differently, some authors count clustered dots as a single dot when individual NORs could not be easily discerned, while others leave off such cells where NORs could not be

easily discerned. This explains for the variation in the values of mean AgNOR count. Crocker et al[53] recommended the counting of 100 cells as a standardised approach to AgNOR dot enumeration and this was followed in our study.

AgNOR AS PROGNOSTIC INDICATOR:

The histological grade, tumour size and the lymph node status forms the most important prognostic indicators for carcinoma of breast.

Kesari AL et al[67], Subramanian S, Shariff S and Karmakar T, Radhika S, Gupta SK[60] and Andrade C[78] proved in their study that tumours showing higher AgNOR counts were of higher grade and are poorly differentiated. Gimenez Mas JA et al[79] and Ofner D et al[80], noted that there was a significant association between tumour grade and the mean AgNOR values, and the AgNOR values increased as the grading increased. However various other authors like Kumar A et al[63], Raymond WA, Leong AS[51] and Gupta GR et al[81], came to a conclusion that no correlation significance was found between tumour grade and mAgNOR values. Kazuhiko Hatano[82] also showed no correlation between mAgNOR count and the grade of the tumour. In his study grade I tumours showed a mAgNOR count of 4.71 +/- 1.17, and grade II tumours showed a mAgNOR count of 4.38 +/- 1.41 and grade III tumours showed a mAgNOR count of 5.42 +/- 1.63.

In our present study 40 breast carcinomas detected by cytology were confirmed by histology and were graded using Bloom and Richardson histological grading system. According to this grading system 5 cases (12.5%) constituted grade I tumours of which all 5 cases were previously graded as Robinson's cytological grade I, 20 cases (50%) constituted grade II tumours among which 17 cases were graded in cytology as Robinson's cytological grade II and the remaining 3 were graded as Robinson's cytological grade I. the remaining 15 cases (37.5%) constituted grade III tumours among which 9 cases had been graded as Robinson's cytological grade II while the remaining 6 were graded as Robinson's cytological grade III.

Kumar A et al. [63] and Gupta GR et al. [81], Gimenez-Mas JA et al.[79] showed that there was a significant association between tumour size and AgNOR values and the mAgNOR count increased as the tumour size increased. Rajeevan K, Aravindan KP and Kumari BC[83] proved that the mean AgNOR count was much higher in tumours greater than 5 cms. Whereas other authors like Hehir DJ et al[84], and Raymond WA, Leong AS[51] noted that the tumour size did not correlate well with the mean AgNOR counts.

In our present study of 40 breast cancers, 2 cases was of T1 size lesions, 34 cases were of T2 size lesions and remaining 4 cases

constituted T3 size lesions. The mean AgNOR count of T1 size lesion was 6+/-0.198, the mean AgNOR count of T2 size lesion was 7.332+/-0.644 and the mean AgNOR count of T3 size lesion was 8.2+/-1.427. These observations showed a gradual increase in mean AgNOR values as the tumour size increased, similar to the findings of the above mentioned studies.

Status of the axillary lymph nodes is one of the most important prognostic indicators of the carcinoma of breast. In our study of 40 cases, 17 cases (42.5%) showed positive lymph node status, while the remaining 23 cases (57.5%) showed negative lymph nodes. The cases with positive lymph nodes expressed a mean AgNOR count of 7.768+/-0.726, while the cases with negative lymph nodes expressed mean AgNOR count of 7.045+/-0.763. These differences in AgNOR values are subtle and are not of much significance as there is only a minor variation. In our study AgNOR values does not correlate much with that of lymph node status as there was overlap with regard to individual values.

Studies conducted by various authors like Karmakar T, Radhika S, Gupta SK[60] , Aubele M, Jutting U, Auer G,[85] Hehir DJ et al,[84] and Raymond WA, Leong AS[51] also confirmed our finding by observing no correlation between lymph node status and AgNOR values.

Study conducted by Simha M, Menon M, Doctor V[62] also proves that no correlation was found between AgNOR values and lymph node status. While studies conducted by Kumar A et al[63], Gupta GR et al[81] Gimenez-Mas JA et al[79] showed a significant correlation between lymph node status and the mean AgNOR values.

SUMMARY

Fine Needle Aspiration Cytology as a preoperative diagnosis is primarily aimed at distinguishing benign from malignant neoplasm. But pathologists are sometimes fraught with diagnostic dilemmas in cytology. In such difficult situations, AgNOR as a proliferative marker is of great help in differentiating benign from malignant neoplasm of the breast.

AgNOR staining as a simple one step silver staining technique can be reliably and effectively utilised to differentiate malignant from benign neoplasm of the breast. The malignant neoplasm consistently showed higher AgNOR values compared to their benign counterparts.

Various studies has shown a wide discrepancy between the absolute value of mean AgNOR counts and the cut off values for differentiating benign from malignant neoplasm of breast. This variation could have stemmed up from the fact that there was a lack of standardisation in the counting of AgNOR dots and also due to inter observer variability.

Our study has tried to prove that AgNORs can reliably be used to differentiate benign non-proliferative lesions from the proliferative lesions of breast, the latter showing consistently higher values compared to the non-proliferative lesions. This is due to the fact that AgNORs act a marker of cellular proliferative activity.

In our study while meticulously examining the AgNOR dots we took into consideration five AgNOR parameters for evaluating the NORs. They are mean AgNOR count, proliferative AgNOR index, AgNOR size variation, AgNOR distribution within the nucleus and the Subjective AgNOR Pattern Assessment (SAPA) score. All these parameters showed consistency in their scoring pattern and showed higher values for malignant tumours compared to the benign ones.

Our study showed very good correlation of all the AgNOR parameters with the Robinson's cytological grading system with regard to malignant neoplasm of breast. All the AgNOR parameters showed a consistent and steady increase in their values as the cytological grade of tumour progressed higher.

Regarding AgNORs being considered as a cell proliferation marker, it showed a consistent and steady increase in the AgNOR values from benign non-proliferative neoplasm to benign proliferative neoplasm and then onto malignant neoplasm of the breast which showed the highest AgNOR value. As a proliferative marker AgNOR is used to predict the biological behaviour of breast neoplasm. In our study the AgNOR parameters did not show a significant correlation with the lymph node status of our patients, but showed a significant correlation with the tumour size and grade of the breast neoplasm. Hence it can be stated that AgNORs can be utilised as a prognostic indicator in the breast neoplasm.

CONCLUSION

Assessment and evaluation of the AgNORs and their parameters in the present study on breast cytology smears has enlightened us to draw the following conclusions-

- ❖ All the AgNOR parameters showed consistently higher values for malignant neoplasm compared to the benign neoplasm of the breast. Mean AgNOR score and SAPA score was found to be more superior compared to other AgNOR parameters and provide additional information in cases that provide diagnostic difficulty in routine FNAC. SAPA score was more reproducible compared to mAgNOR count.
- ❖ AgNORs are helpful in differentiating benign proliferative neoplasm from non-proliferative neoplasm of breast. The proliferative neoplasm showed higher AgNOR values compared to the non-proliferative neoplasm of the breast.
- ❖ AgNOR showed a good correlation with the Robinson's cytological grading system and the AgNOR values increased as the cytological grade of the neoplasm progressed higher.
- ❖ As cell proliferative marker mAgNOR count and SAPA score represent proliferating cellular activity and hence predict the biological behaviour of the breast neoplasm.

FIBROCYSTIC DISEASE

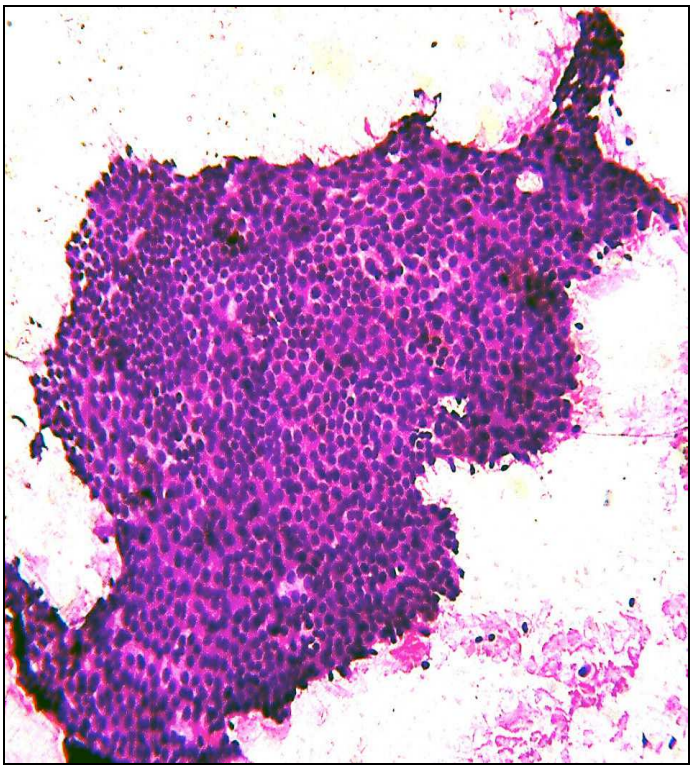


Figure 3 Aspirate of fibrocystic change showing honeycombing of uniform ductal epithelial cells with bipolar naked nuclei in the background (H&E, 100X)

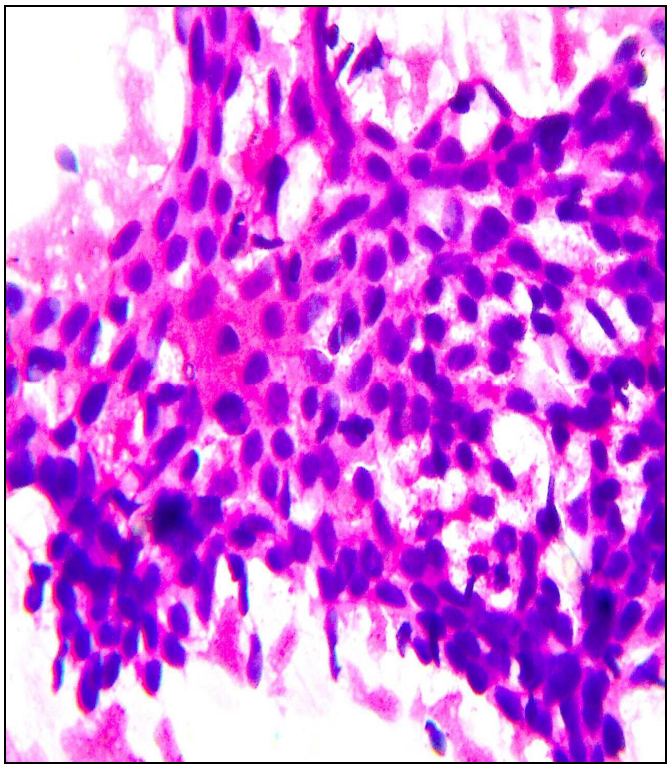


Figure 4 Uniform duct epithelial cell clusters in fibrocystic change of breast (H&E, 400X)

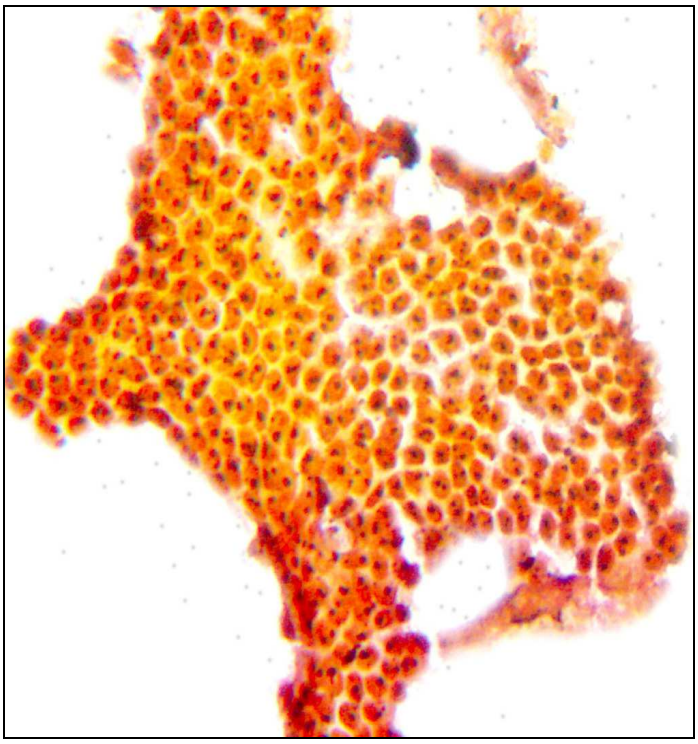


Figure 5 Duct epithelial cell clusters in honeycomb arrangement showing only a few AgNOR dots (AgNOR, 400X)

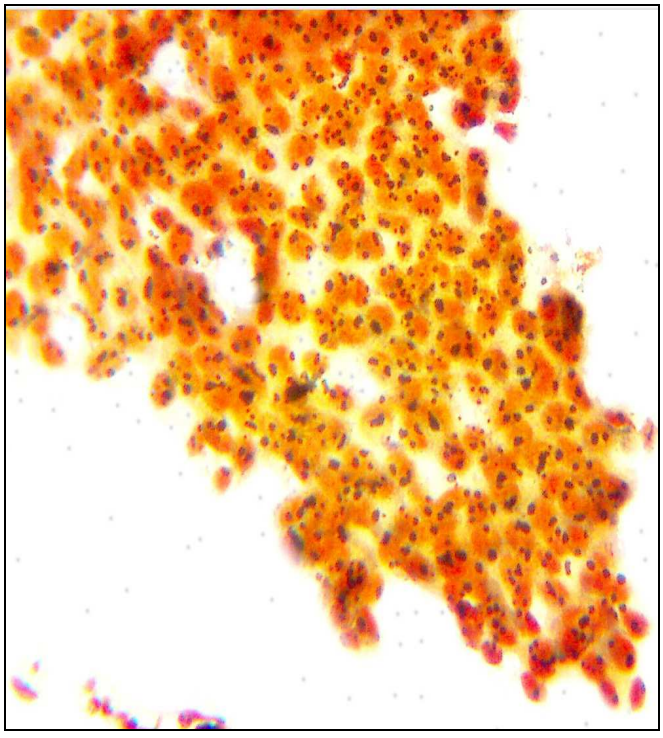


Figure 6 Uniform duct epithelial cell clusters showing only a few AgNOR dots (AgNOR, 400X)

EPITHELIAL HYPERPLASIA

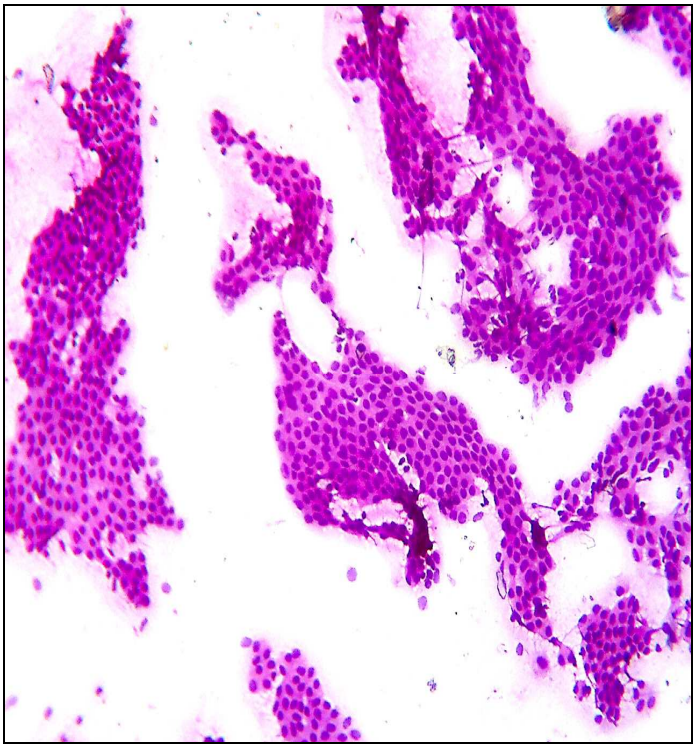


Figure 7 Large slightly disorganised sheets of hyperplastic duct epithelial cell clusters with tendency to streaming (H&E, 100X)

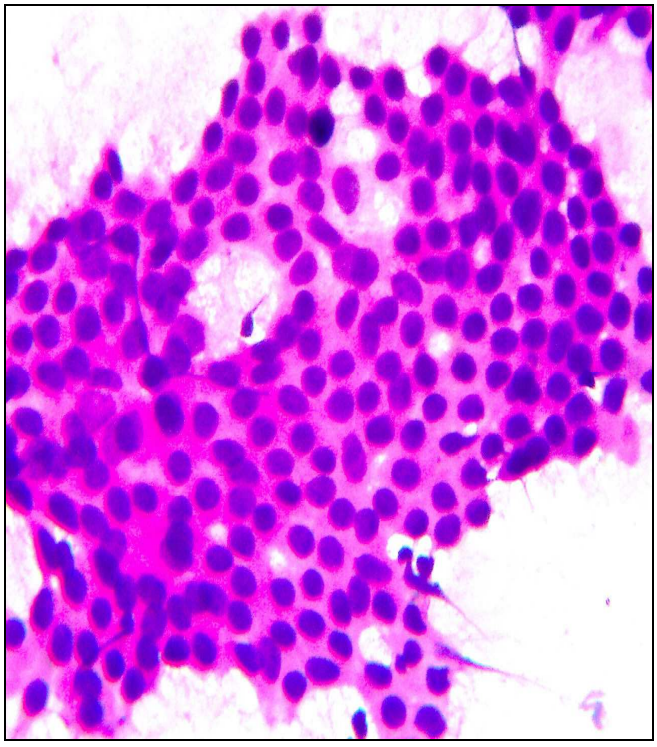


Figure 8 Large sheet of hyperplastic duct epithelial cell clusters (H&E, 400X)

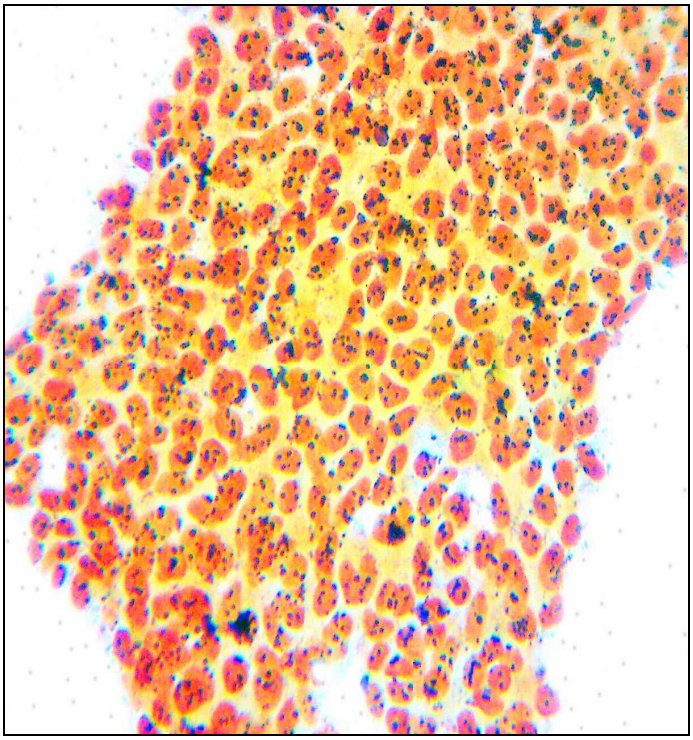


Figure 9 Large sheets of hyperplastic duct epithelial cell clusters showing 3-4 AgNOR dots per nucleus (AgNOR, 400X)

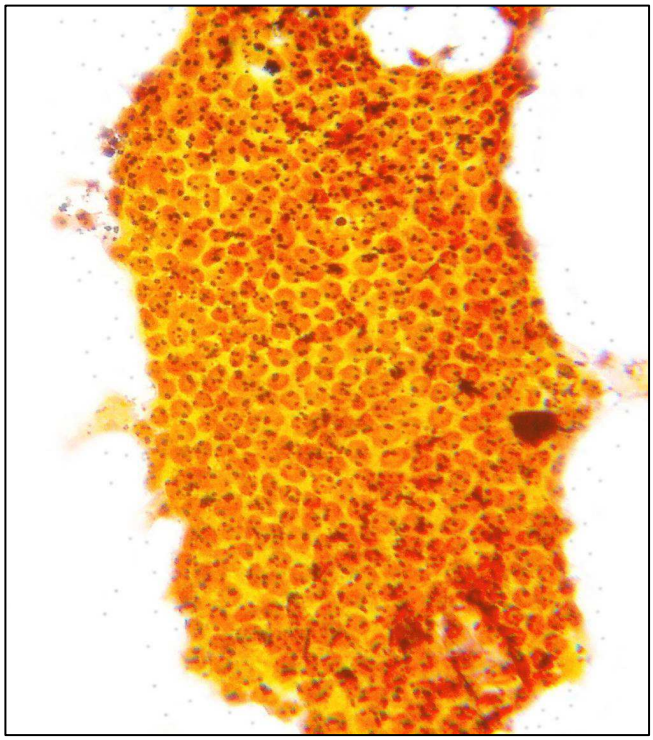


Figure 10 Hyperplastic duct epithelial cells in cohesive clusters showing 3-4 AgNOR dots per nucleus (AgNOR, 400X)

ATYPICAL DUCTAL HYPERPLASIA

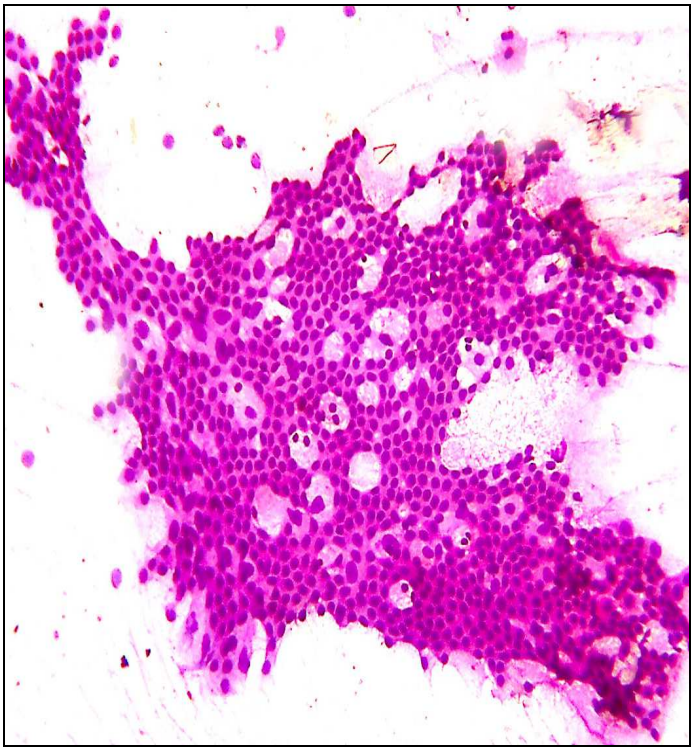


Figure 11 Large sheets of mildly atypical duct epithelial cells with holes indicating a cribriform pattern (H&E, 100X)

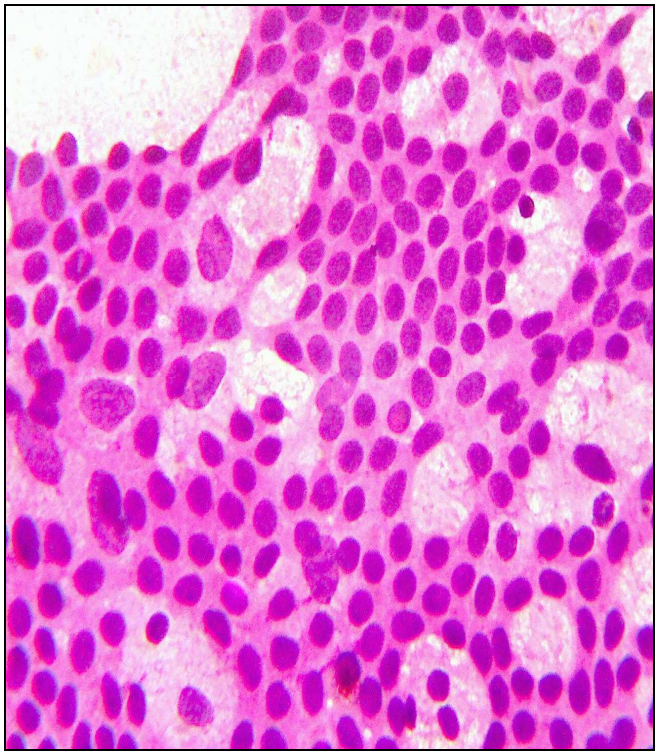


Figure 12 Large slightly disorganised sheet of mildly atypical duct epithelial cells (H&E, 400X)

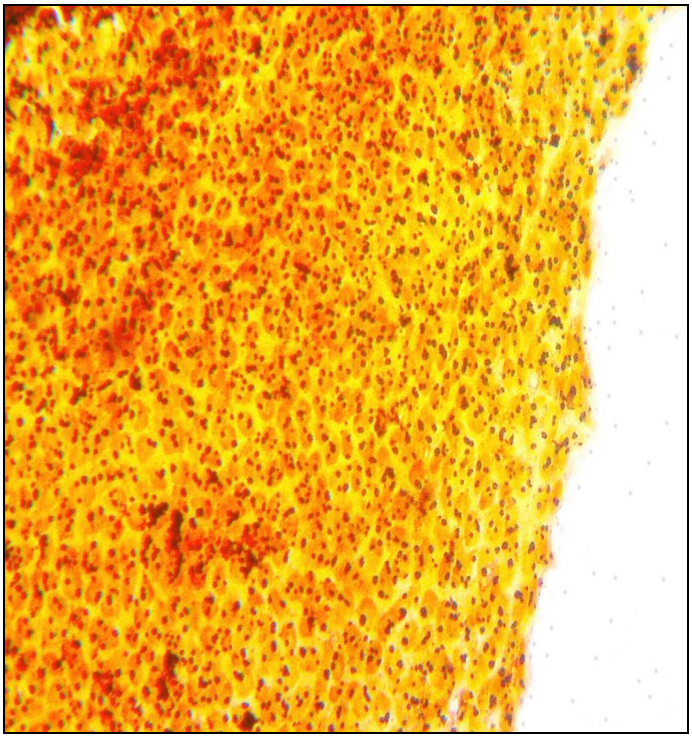


Figure 13 Atypical hyperplastic duct epithelial cell cluster of breast showing 4-5 AgNOR dots per nucleus (AgNOR, 400X)

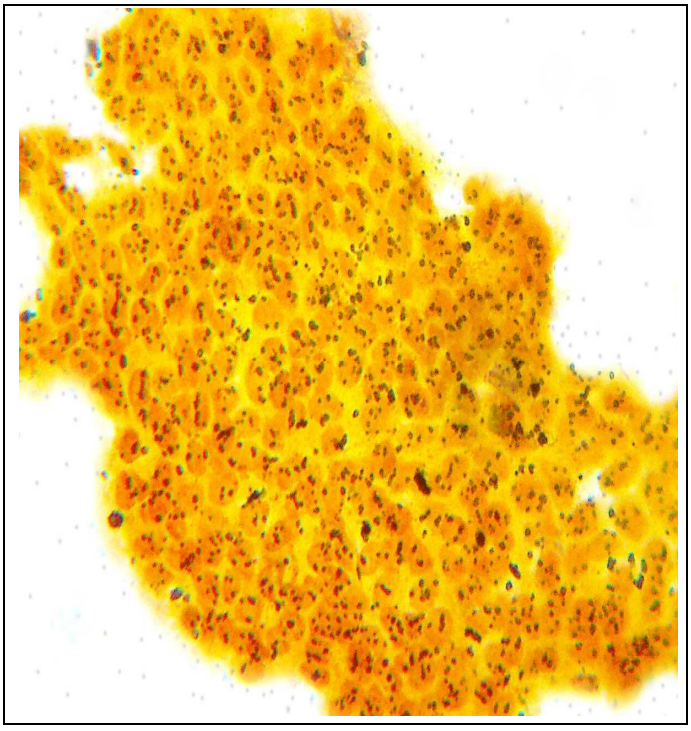


Figure 14 Cluster of hyperplastic duct epithelial cells with atypia showing 4-5 AgNOR dots per nucleus (AgNOR, 400X)

INTRADUCTAL PAPILLOMA

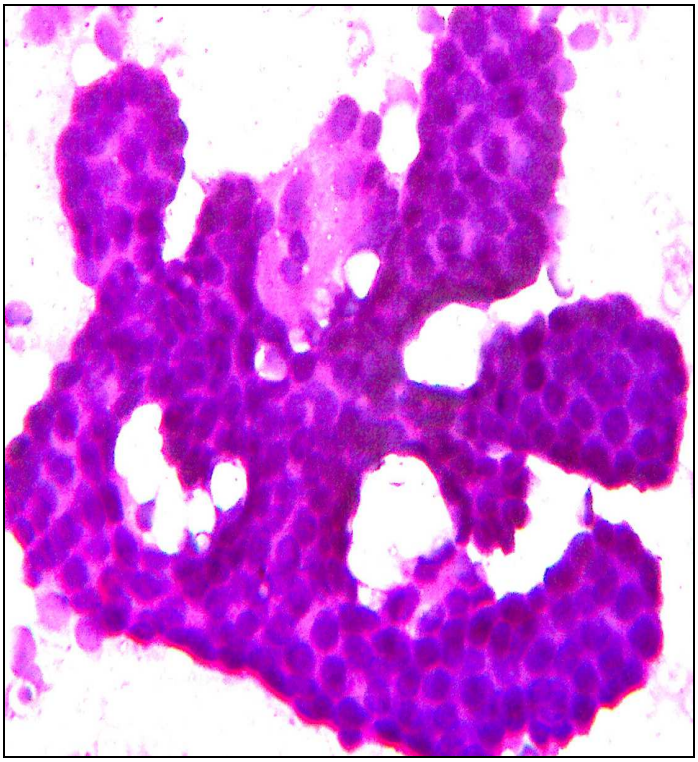


Figure 15 Finger like papillary structures seen in intraductal papilloma of breast (H&E, 400X)

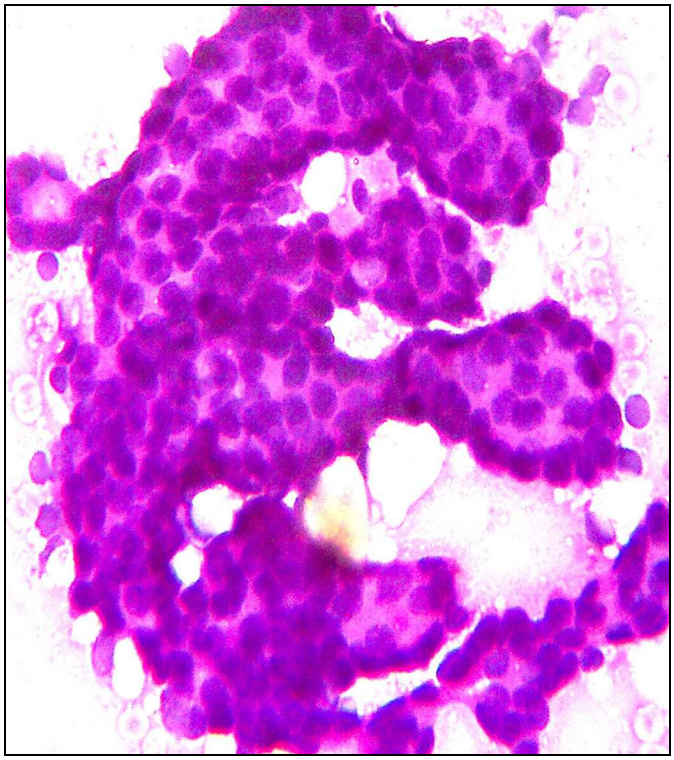


Figure 16 Papillary fragments of the duct epithelial cells of breast (H&E, 400X)

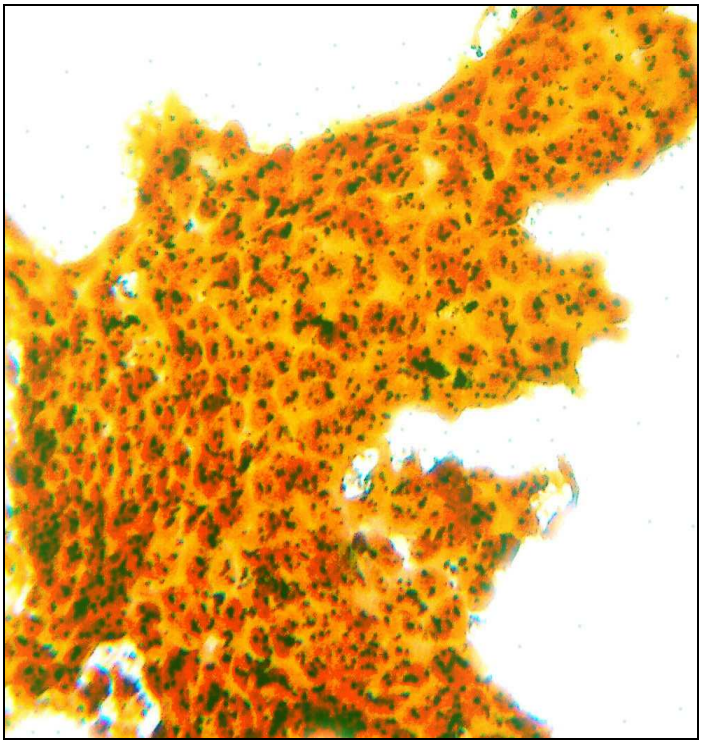


Figure 17 Finger like papillary structures of breast showing 4-5 AgNORs per nucleus (AgNOR, 400X)

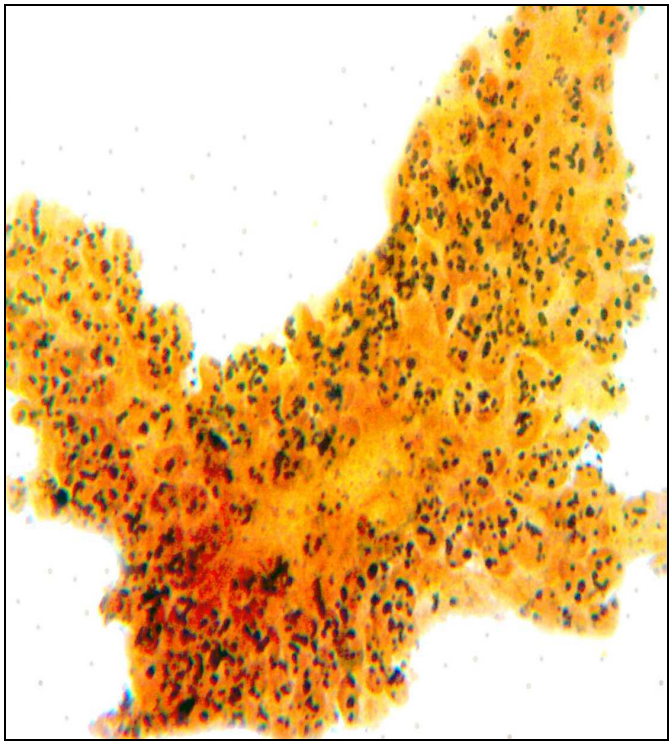


Figure 18 Papillary structures with fibrovascular core showing 4-5 AgNORs per nucleus (AgNOR, 400X)

BREAST CARCINOMA – GRADE 1

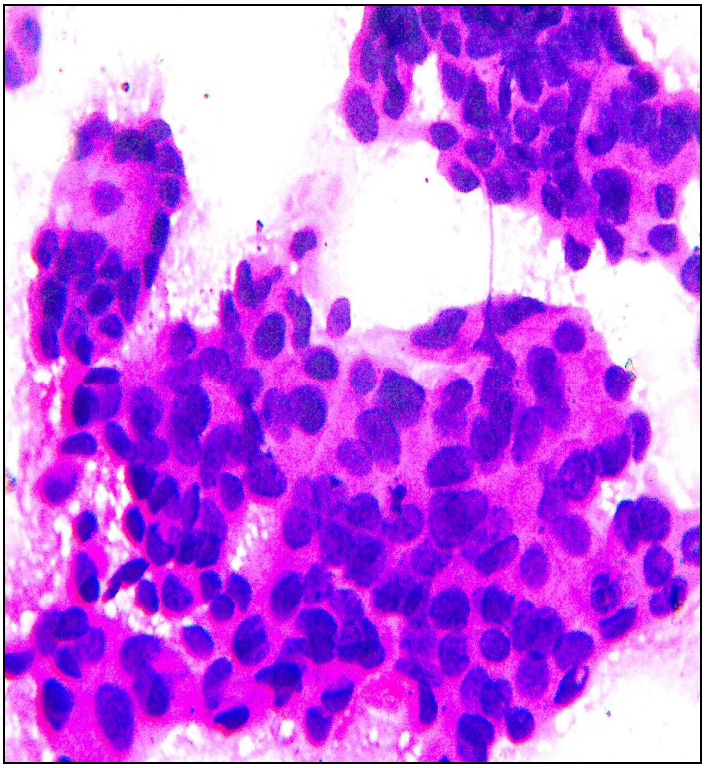


Figure 19 Cell rich dyscohesive clusters of epithelial cells with mild atypia of nucleus (H&E. 400X)

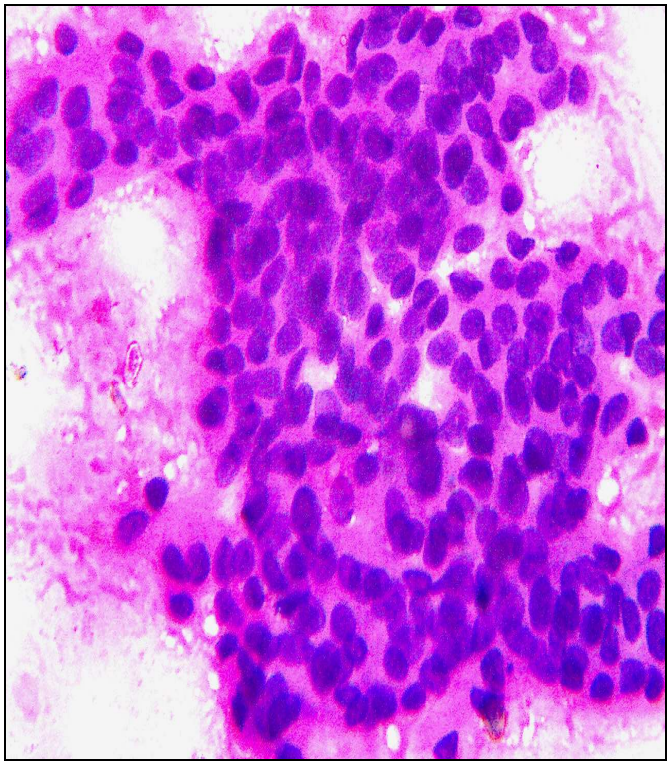


Figure 20 Dyscohesive clusters of epithelial cells with mild cellular pleomorphism with nuclear overlapping (H&E, 400X)

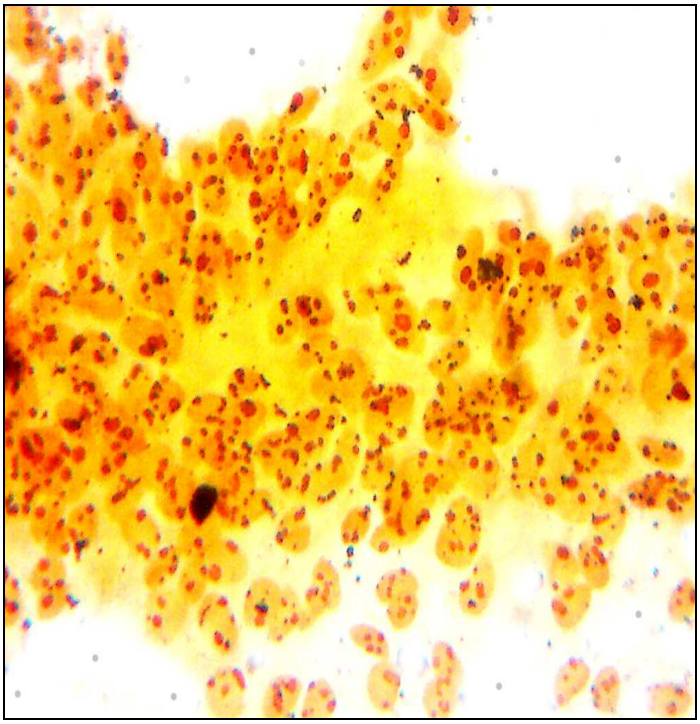


Figure 21 Dyscohesive clusters of pleomorphic duct epithelial cells showing increased number of AgNORs per nucleus (AgNOR, 400X)

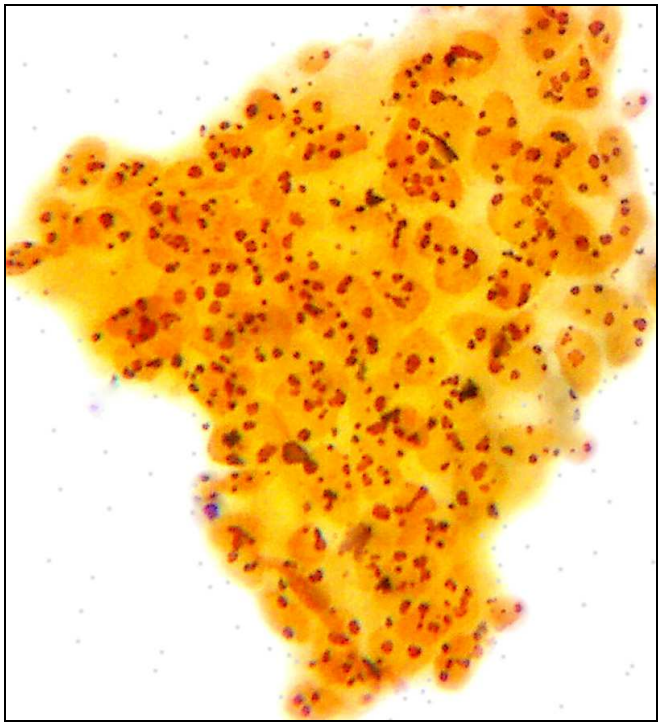


Figure 22 Clusters of duct epithelial cells with mild nuclear pleomorphism showing increased number of AgNORs per nucleus (AgNOR, 400X)

BREAST CARCINOMA – GRADE 2

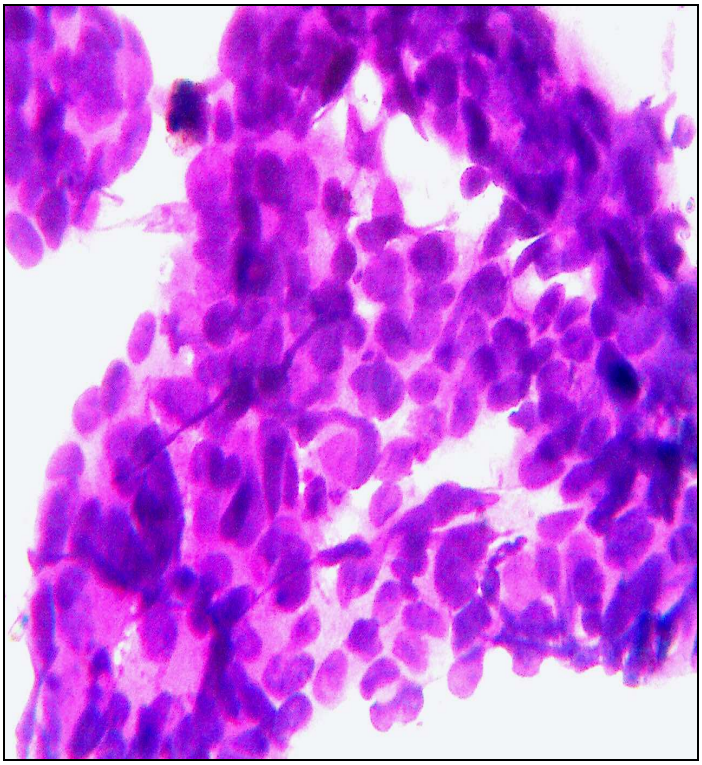


Figure 23 Dyscohesive clusters of epithelial cells with moderate nuclear pleomorphism (H&E, 400X)

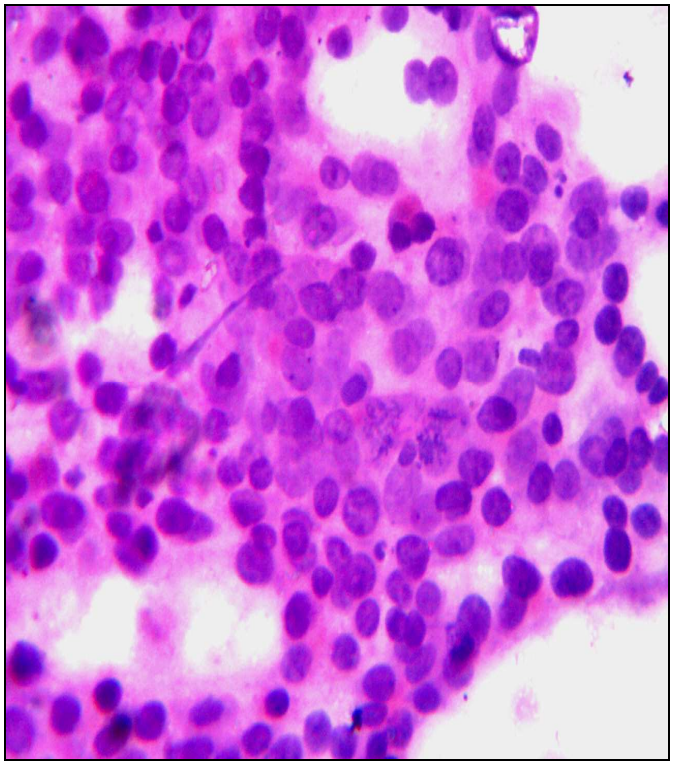


Figure 24 Dyscohesive clusters of epithelial cells with moderate nuclear enlargement and atypia (H&E, 400X)

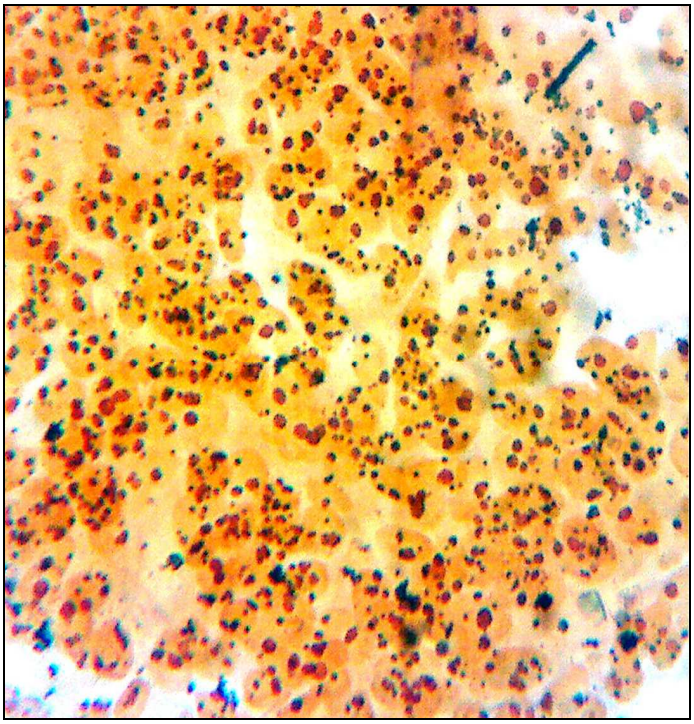


Figure 25 Clusters of duct epithelial cells with moderate nuclear pleomorphism showing higher number of AgNORs per nucleus (AgNOR, 400X)

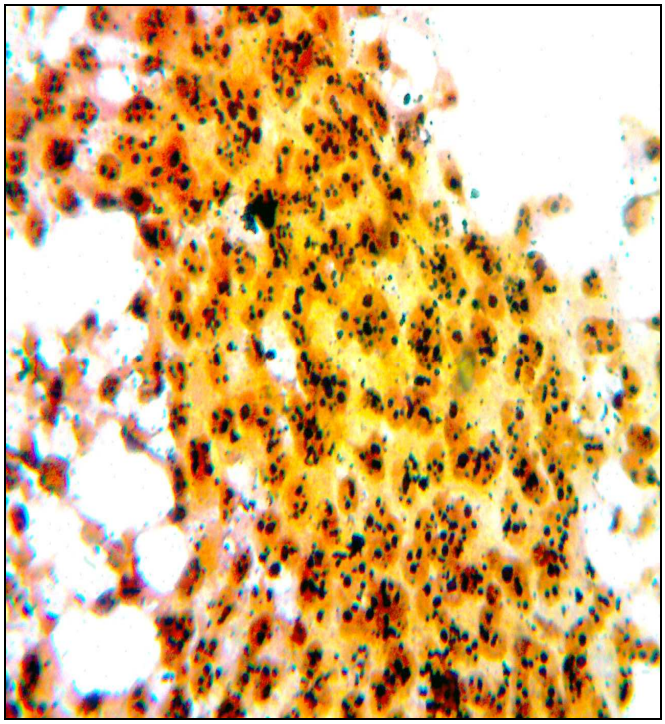


Figure 26 Dyscohesive clusters of epithelial cells with moderate nuclear atypia showing higher number of AgNORs per nucleus (AgNOR, 400X)

BREAST CARCINOMA – GRADE 3

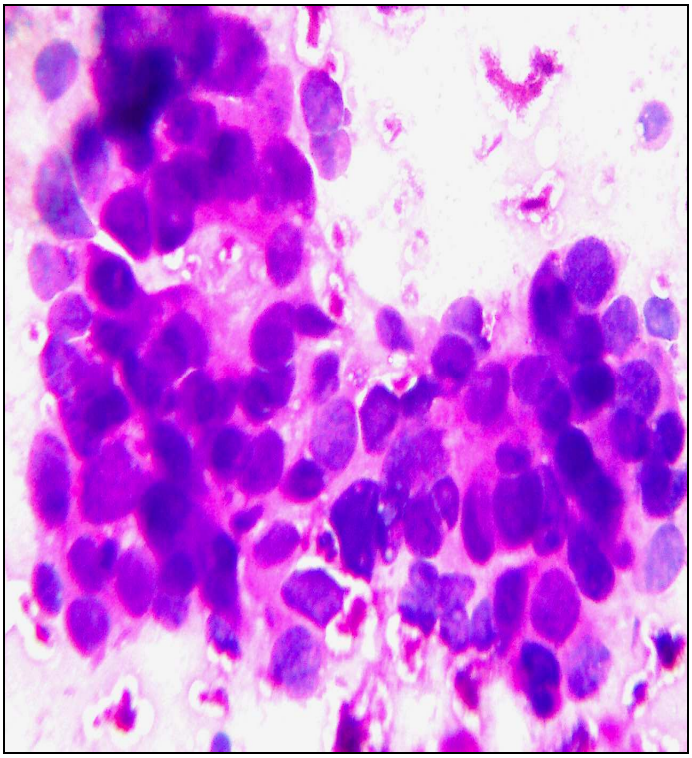


Figure 27 Poorly cohesive clusters of duct epithelial cells showing nuclear enlargement and pleomorphism (H&E, 400X)

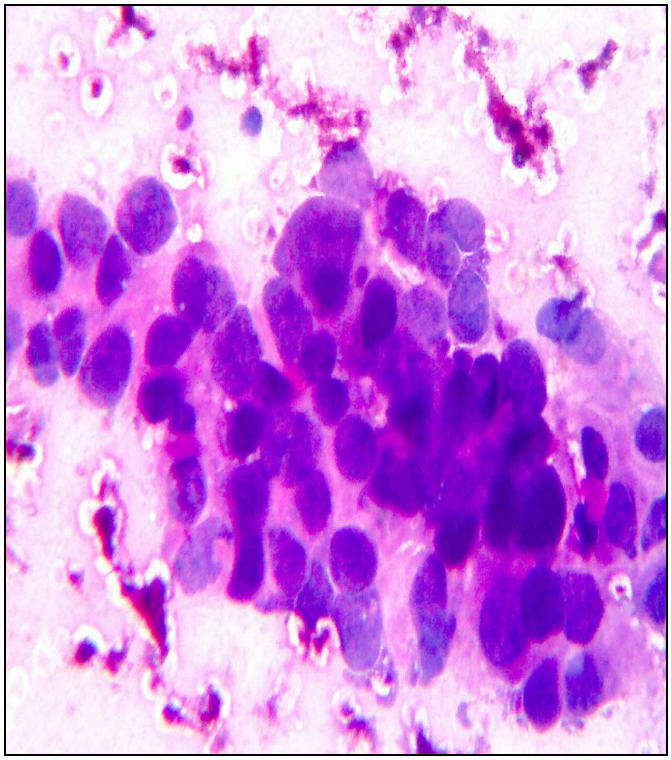


Figure 28 Highly dyscohesive clusters of duct epithelial cells showing obvious nuclear enlargement & overlapping (H&E, 400X)

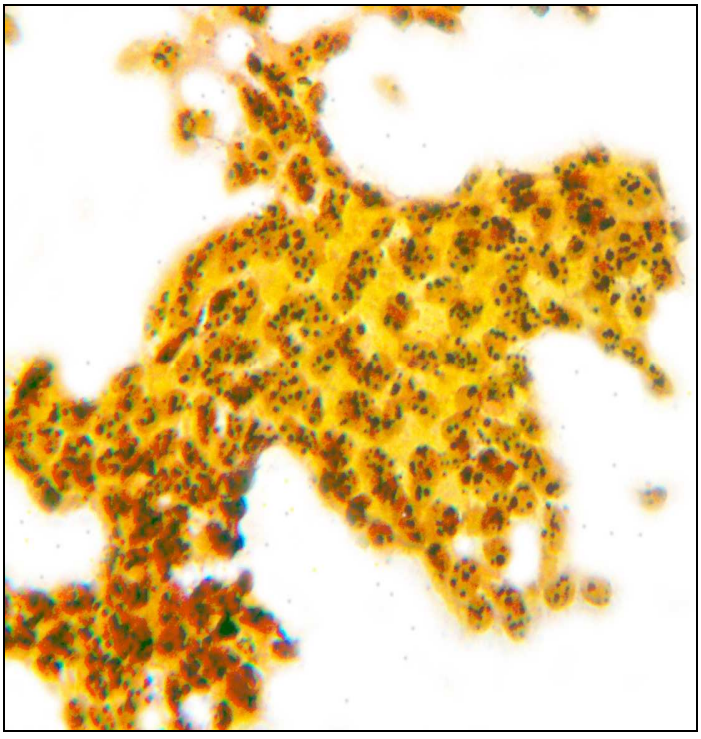


Figure 29 Poorly cohesive clusters of duct epithelial cells showing showing numerous AgNORs per nucleus (AgNOR, 400X)

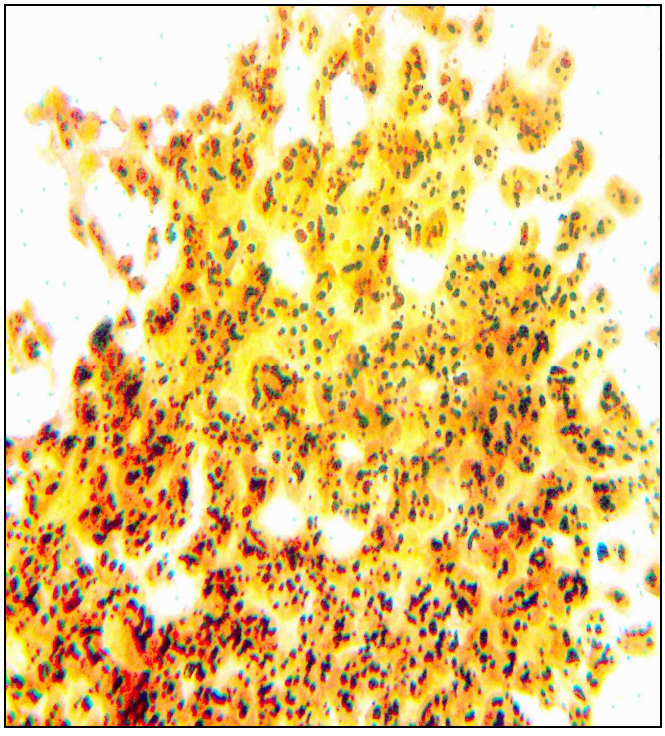


Figure 30 Highly dyscohesive clusters of duct epithelial cells showing numerous AgNORs per nucleus (AgNOR, 400X)

SL.NO	I.P. No	FNAC. No	AGE	SEX	SIDE	QUADRANT	SIZE	FNAC DIAGNOSIS	LN STATUS	AgNOR SCORE					ROBINSON SCORE	ROBINSON GRADE	HISTOPATH DIAGNOSIS
										mAgNOR	pAgNOR	AgNOR SIZE	AgNOR DIS	SAPA			
1	82531	870/11	38	F	R	LOQ	4	DC	NEG	7.41	58	2	2	7	13	II	DC-II
2	82577	872/11	58	F	R	UIQ	4	DC	POS	7.56	51	2	2	7	13	II	DC-III
3	23654	1008/11	33	F	R	LOQ	1	EPI		4.68	34	1	1	4			
4	96254	1110/11	50	F	L	UOQ	0.5	FCD		2.87	18	1	1	3			
5	123917	1383/11	50	F	R	UIQ	5	DC	POS	8.54	62	3	3	8	17	III	DC-III
6	36244	1541/11	65	F	R	LOQ	5	DC	POS	7.94	49	3	3	8	16	III	DC-III
7	139309	1544/11	37	F	L	LOQ	2.5	ADH		4.92	34	1	1	4			
8	37421	1574/11	50	F	L	LOQ	4.5	DC	POS	8.71	49	3	3	8	14	II	DC-III
9	171607	1881/11	42	F	L	UOQ	4	DC	POS	7.57	48	2	2	7	12	II	DC-II
10	188019	2060/11	69	F	L	LOQ	1	FCD		1.86	11	0	0	3			
11	50573	2088/11	56	F	R	UOQ	5.5	DC	POS	8.52	47	3	3	8	14	II	DC-III
12	196648	2093/11	49	F	L	LOQ	4	DC	NEG	7.63	50	2	2	7	12	II	DC-III
13	50958	2100/11	49	F	R	UIQ	3.5	DC	POS	6.87	45	2	2	6	12	II	DC-II
14	53933	2205/11	59	F	R	UIQ	4.5	DC	POS	7.07	49	2	2	6	12	II	DC-II
15	212201	2211/11	40	F	R	UOQ	1.5	EPI		3.79	28	1	1	4			
16	211989	2216/11	65	F	R	UOQ	2	ADH		3.87	24	1	1	3			
17	2186	028/12	50	F	L	LOQ	1	EPI		3.83	21	1	1	4			
18	2913	087/12	26	F	L	UOQ	0.5	FAD		1.71	6	0	0	3			
19	12458	114/12	42	F	R	UOQ	2	ADH		4.86	38	1	1	4			
20	11490	127/12	42	F	L	UOQ	4	DC	NEG	7.97	55	2	2	7	14	II	DC-III
21	17667	164/12	62	F	L	LIQ	4	DC	NEG	7.31	52	2	2	7	13	II	DC-II
22	14100	175/12	38	F	L	UOQ	1	FAD		1.91	10	0	0	3			
23	19301	179/12	45	F	L	UOQ	4.5	DC	NEG	7.04	48	2	2	6	10	I	DC-II
24	19905	197/12	44	F	R	LOQ	1	EPI		5.35	41	2	2	5			
25	6210	205/12	62	F	R	UOQ	4	DC	NEG	7.67	58	2	2	7	13	II	DC-III
26	23655	256/12	23	F	L	LIQ	1.5	EPI		6.21	51	2	2	5			
27	8403	264/12	32	F	R	LOQ	1.5	FCD		2.17	13	0	0	3			
28	8638	276/12	71	F	L	UOQ	5	DC	POS	7.36	52	2	2	7	10	I	DC-II
29	24858	278/12	57	F	R	UOQ	4.5	DC	NEG	7.87	54	3	3	7	15	III	DC-III
30	9216	289/12	30	F	R	UOQ	2	EPI		3.82	31	1	1	4			
31	8105	298/12	44	F	R	LOQ	7	DC	POS	9.54	71	3	3	8	17	III	DC-III
32	8644	313/12	52	F	R	UOQ	3.5	DC	POS	7.31	53	2	2	6	13	II	DC-II
33	10163	323/12	56	F	L	UOQ	2.5	ADH		4.92	42	1	1	4			
34	31292	350/12	19	F	L	UOQ	1.5	EPI		4.65	36	1	1	5			
35	31709	361/12	37	F	L	UIQ	1	FAD		2.33	11	0	0	3			
36	11231	365/12	43	F	L	UOQ	4.5	DC	NEG	6.94	47	2	2	6	9	I	DC-I
37	11449	384/12	40	F	L	UOQ	4.5	DC	NEG	8.04	59	3	3	7	14	II	DC-III
38	35836	404/12	36	F	L	LOQ	1.5	ADH		4.99	37	1	1	5			
39	38464	409/12	33	F	R	UOQ	1	EPI		4.47	33	1	1	4			
40	38602	422/12	23	F	L	LOQ	1	FAD		2.13	9	0	0	3			
41	41510	445/12	56	F	L	UOQ	4	DC	NEG	6.89	49	2	2	6	13	II	DC-II

42	41469	446/12	56	F	L	LOQ	1.5	DC	NEG	5.86	40	2	2	6	7	I	DC-I
43	13470	450/12	45	F	L	UOQ	4	DC	POS	7.02	50	2	2	6	13	II	DC-II
44	41439	456/12	62	F	L	UOQ	4	DC	NEG	7.17	51	2	2	7	13	II	DC-II
45	42414	462/12	55	F	R	LOQ	1.5	DC	NEG	6.14	43	2	2	6	8	I	DC-I
46	40338	476/12	18	F	L	CQ	2	IDP		4.86	39	1	1	4			
47	13880	518/12	35	F	L	LIQ	1.5	FCD		1.74	4	0	0	3			
48	48252	542/12	43	F	R	UOQ	1	EPI		4.14	32	1	1	4			
49	50720	557/12	19	F	R	UOQ	1.5	EPI		4.12	29	1	1	4			
50	50982	570/12	38	F	L	LOQ	1.5	FCD		2.96	23	0	0	3			
51	51876	575/12	19	F	L	UOQ	1	FAD		3.06	21	0	1	3			
52	56990	646/12	24	F	L	UIQ	0.5	FCD		2.36	4	0	0	3			
53	58771	669/12	45	F	R	UOQ	0.5	FCD		2.94	20	0	1	3			
54	52642	685/12	42	F	L	LOQ	1	FCD		3.11	18	1	1	3			
55	61177	693/12	50	F	L	LOQ	5	DC	POS	7.9	54	3	3	8	16	III	DC-III
56	63402	698/12	42	F	L	UOQ	1	FCD		1.83	3	0	0	3			
57	17333	701/12	60	F	R	LOQ	5	DC	POS	8.14	60	3	3	8	16	III	DC-III
58	62682	710/12	17	F	L	UOQ	1.5	FAD		2.13	9	0	0	3			
59	63875	718/12	39	F	L	LOQ	3.5	DC	POS	7.06	49	2	2	6	12	II	DC-II
60	65718	741/12	30	F	L	LOQ	1	FCD		3.18	14	1	1	3			
61	22731	745/12	17	F	L	UOQ	1.5	EPI		3.92	27	1	1	4			
62	65894	746/12	42	F	R	LOQ	4.5	DC	POS	7.67	52	2	2	7	12	II	DC-III
63	69993	775/12	62	F	R	UIQ	3.5	DC	NEG	7.27	46	2	2	7	10	I	DC-II
64	17995	779/12	30	F	R	UIQ	2	ADH		4.17	29	1	1	4			
65	70025	786/12	39	F	R	LOQ	1.5	ADH		5.16	42	1	1	5			
66	50206	794/12	40	F	L	LOQ	3.5	DC	NEG	6	47	2	2	6	12	II	DC-II
67	25141	809/12	45	F	R	LOQ	4	DC	NEG	7.61	58	2	2	7	14	II	DC-II
68	25223	810/12	42	F	R	UOQ	2.5	ADH		4.86	33	1	1	4			
69	25617	823/12	34	F	R	UOQ	3.5	DC	NEG	7.09	47	2	2	6	13	II	DC-II
70	25894	834/12	45	F	R	UOQ	3.5	DC	NEG	5.8	40	2	2	6	8	I	DC-I
71	71968	840/12	37	F	L	LOQ	1	FAD		3.24	25	0	0	3			
72	26611	863/12	66	F	L	CQ	2	IDP		5.83	42	2	1	5			
73	73779	871/12	35	F	R	LIQ	1	FCD		1.67	14	0	0	3			
74	71349	879/12	33	F	R	UOQ	1	EPI		5.97	42	2	2	5			
75	76921	902/12	43	F	R	LIQ	0.5	FAD		3.35	22	1	1	4			
76	75008	904/12	20	F	L	LOQ	0.5	FAD		3.32	23	1	1	3			
77	78731	913/12	40	F	R	LOQ	1	FAD		2.01	6	0	0	3			
78	79015	933/12	40	F	L	UIQ	1.5	ADH		5.23	43	2	2	5			
79	82892	992/12	24	F	R	UOQ	0.5	FAD		2.34	11	0	0	3			
80	85138	1026/12	25	F	R	LOQ	0.5	FAD		1.85	9	0	0	3			
81	23237	1027/12	27	F	L	UOQ	0.5	FAD		2.09	6	0	0	3			
82	88430	1054/12	40	F	R	UOQ	6	DC	NEG	8.56	62	3	3	8	14	II	DC-III
83	89371	1064/12	25	F	L	UIQ	0.5	FCD		2.19	10	0	0	3			
84	89930	1084/12	70	F	L	LOQ	4	DC	NEG	6.74	47	2	2	7	13	II	DC-II
85	93242	1126/12	40	F	R	UOQ	2	ADH		5.12	38	1	1	5			
86	36973	1159/12	44	F	L	UOQ	5.5	DC	NEG	6.18	41	2	2	6	12	II	DC-II
87	36931	1162/12	53	F	R	UOQ	4.5	DC	POS	7.28	54	2	2	7	14	II	DC-II

88	37791	1204/12	40	F	L	LOQ	5	DC	NEG	6.71	48	2	2	6	13	II	DC-II
89	100702	1227/12	35	F	R	UOQ	1.5	EPI		4.52	33	1	1	5			
90	109385	1318/12	48	F	L	UOQ	3.5	DC	NEG	6.14	40	2	2	6	9	I	DC-I
91	162046	1390/12	43	F	L	UOQ	1	FAD		2.51	16	0	0	3			
92	163537	1394/12	47	F	L	LOQ	1	FAD		1.84	7	0	0	3			
93	172702	1514/12	34	F	R	LIQ	1.5	FAD		2.29	8	0	0	3			
94	110902	1553/12	34	F	L	UOQ	1.5	EPI		2.78	19	1	1	4			
95	111586	1602/12	35	F	R	UOQ	1	EPI		3.72	18	1	1	4			
96	191355	1721/12	40	F	L	LOQ	1	FAD		2.09	9	0	0	3			
97	191213	1723/12	45	F	R	UOQ	1.5	EPI		4.67	32	1	1	4			
98	191736	1735/12	38	F	L	UIQ	2	EPI		3.86	24	1	1	4			
99	111858	1744/12	28	F	R	UIQ	1.5	EPI		5.05	40	1	1	5			
100	203626	1900/12	22	F	R	UOQ	2	ADH		5.83	44	2	2	5			

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APPENDIX I

AgNOR STAINING PROCEDURE

METHOD:

AgNOR staining was performed using a one step silver – colloid technique.

PREPARATION OF STAINING SOLUTION:

Solution A: 2% gelatin in 1% formic acid

Solution B: 50% aqueous silver nitrate solution

WORKING SOLUTION:

One part of solution A mixed with two parts of solution B.

STAINING METHODS:

- The aspirated material is smeared onto the slides and is fixed immediately in 95% ethanol.
- The slides are then stained subsequently with AgNOR stain.
- The working solution mixture A & B are layered over the slides and are kept in a dark room for a period of 50 – 60 minutes.
- The silver colloid was then washed off with deionised water.
- The smears are then dehydrated through alcohol.
- Cleared in Xylene.
- Mounted using DPX mounting medium.

APPENDIX II

HEMATOXYLIN AND EOSIN STAINING PROCEDURE

1. The aspirated material is smeared onto the slides and is fixed immediately in 95% ethanol.
2. Stain in alum haematoxylin for 7 min
3. Wash well in running tap water.
4. Differentiate in acid alcohol for 5 seconds
5. Wash well in running tap water
6. Stain in 1% Eosin Y for 3minutes
7. Wash in running tap water for 5minutes
8. Dehydrate through graded alcohols
9. Clear in Xylene.
10. Mount using DPX mounting medium

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APPENDIX III

"VALUE OF ROBINSON'S SCORING SYSTEM AND AgNOR SCORE IN CLASSIFICATION OF PROLIFERATIVE & MALIGNANT EPITHELIAL BREAST DISEASES ON FNAC"

PROFORMA

FNAC NO: _____ . **IP/OP. NO. : _____ .**

PATIENT NAME: _____.

AGE: _____ . **SEX: M / F** **UNIT/WARD: _____.**

ADDRESS: _____ .

CLINICAL DIAGNOSIS: _____.

I) PRESENTING COMPLAINTS: _____ .

II) PERSONAL HISTORY: _____.

III) FAMILY HSITORY: _____ .

IV) GENERAL EXAMINATION:_____ .

VI) LOCAL EXAMINATION: _____

_____.

VII) FNAC DIAGNOSIS: _____.

VIII) ROBINSON'S SCORE: _____.

IX) HPE DIAGNOSIS: _____ .

X) mAgNOR VALUE: _____.

XI) pAgNOR VALUE: _____.

XII) SAPA SCORE: _____.

XIII) AgNOR SIZE VARIATION: _____.

XIV) AgNOR DISTRIBUTION: _____.

KEY TO MASTER CHART

ADH	–	Atypical Ductal Hyperplasia
AgNOR	–	Argyrophilic Nucleolar Organizer Region
AgNOR Dis	–	AgNOR distribution
CQ	–	Central quadrant
DC	–	Ductal carcinoma
EPI	–	Epithelial Hyperplasia
F	–	Female
FAD	–	Fibroadenosis
FCD	–	Fibrocystic disease
IDP	–	Intra Ductal Papilloma
L	–	Left Side
LIQ	–	Lower Inner Quadrant
LN	–	Lymph Node status
LOQ	–	Lower Outer Quadrant
m AgNOR	–	Mean AgNOR
NEG	–	Negative
pAgNOR	–	Proliferative AgNOR index
POS	–	Positive
R	–	Right side
SAPA	–	Subjective AgNOR Pattern Assessment
UIQ	–	Upper Inner Quadrant
UOQ	–	Upper Outer Quadrant